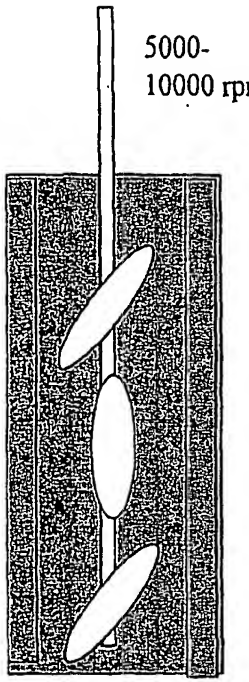
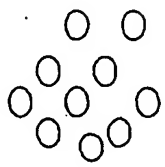
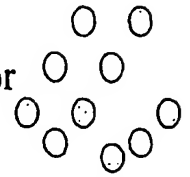
Scheme 1 A



Labelling and immobilisation of microbeads by aqueous inverse suspension polymerization

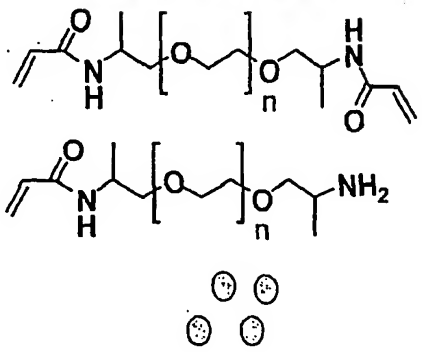


Labelling with
2,3-dinaphtaldehyde or
fluorescein
succinimide ester

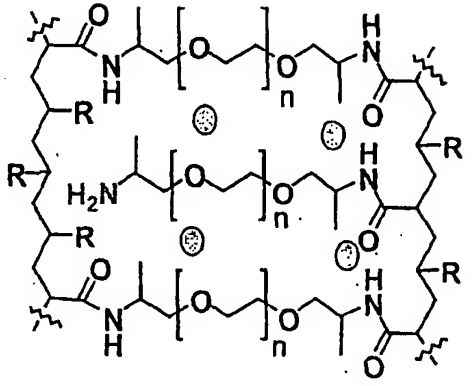


5-10 micron
PEGA beads

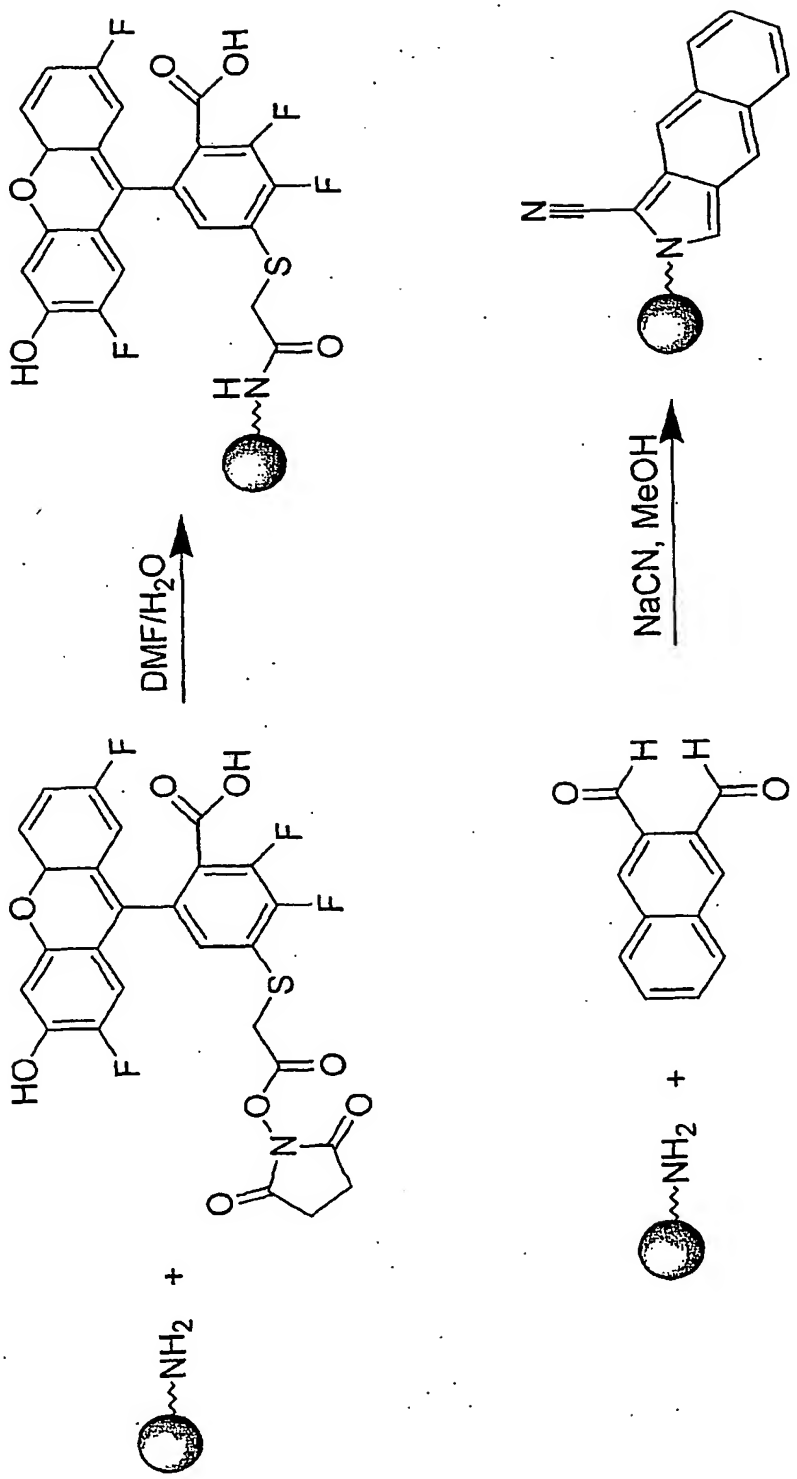
5-10 micron
Labelled beads



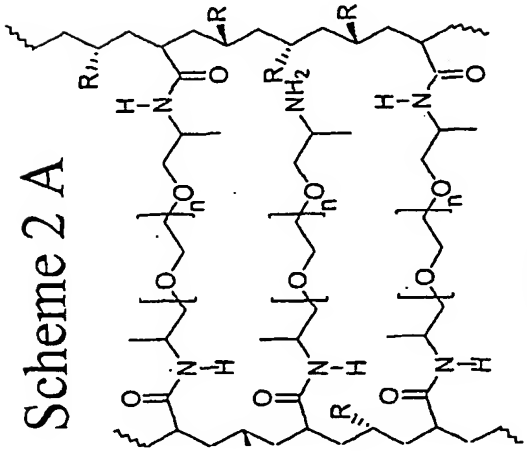
Inverse suspension
polymerization
with particle
immobilisation



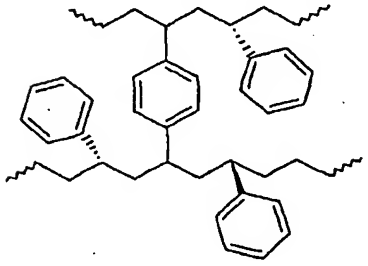
Scheme 1 B



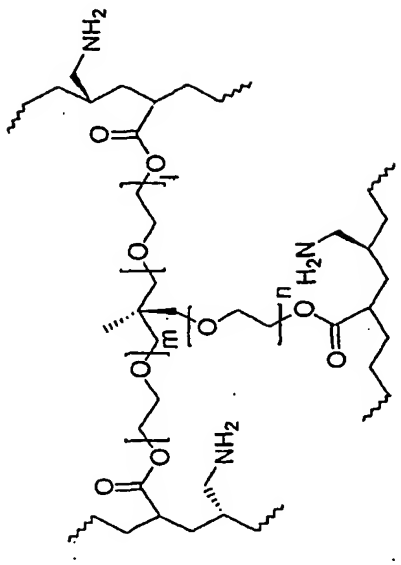
Scheme 2 A



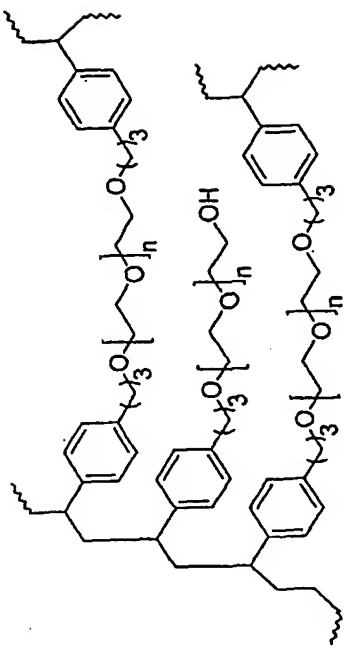
PEGA



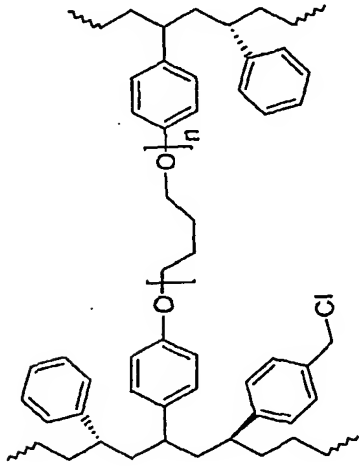
PS-DVB



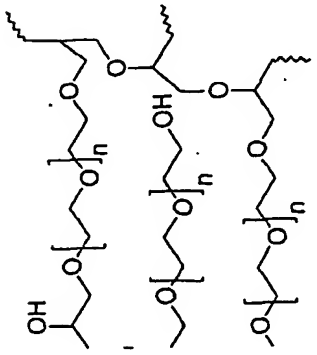
CLEAR



POEPS-3

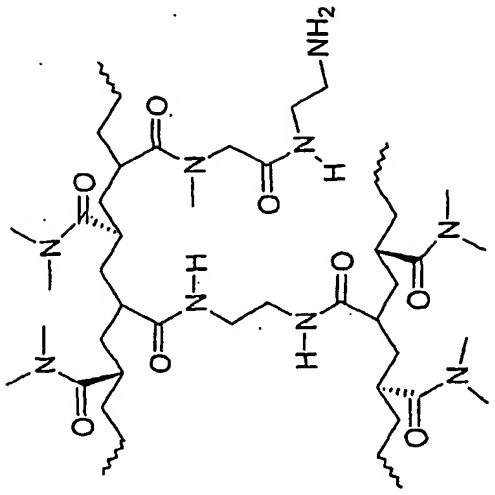


JANDAGEL

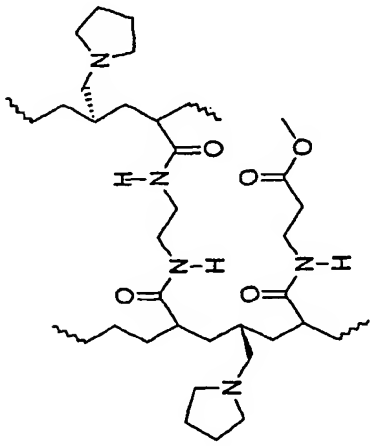


POEPOP

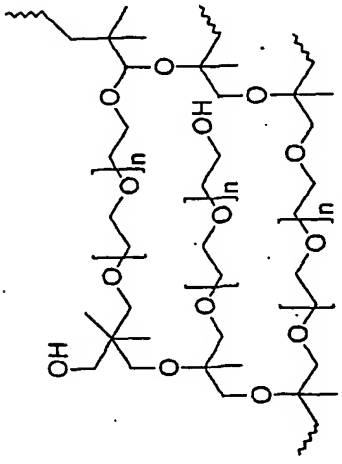
Scheme 2 B



POLYAMIDE



EXPANSIN



SPOCC

Fig. 1

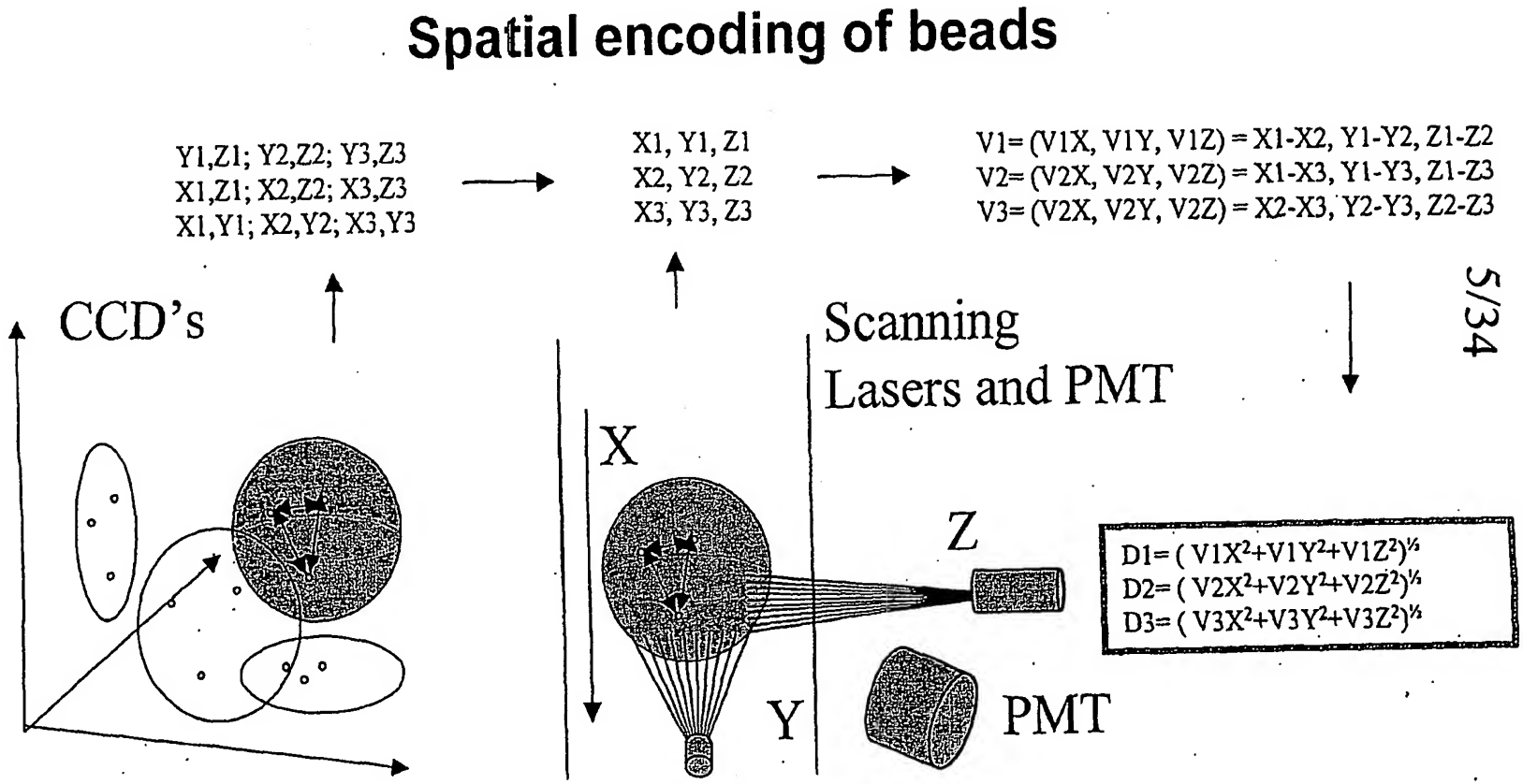
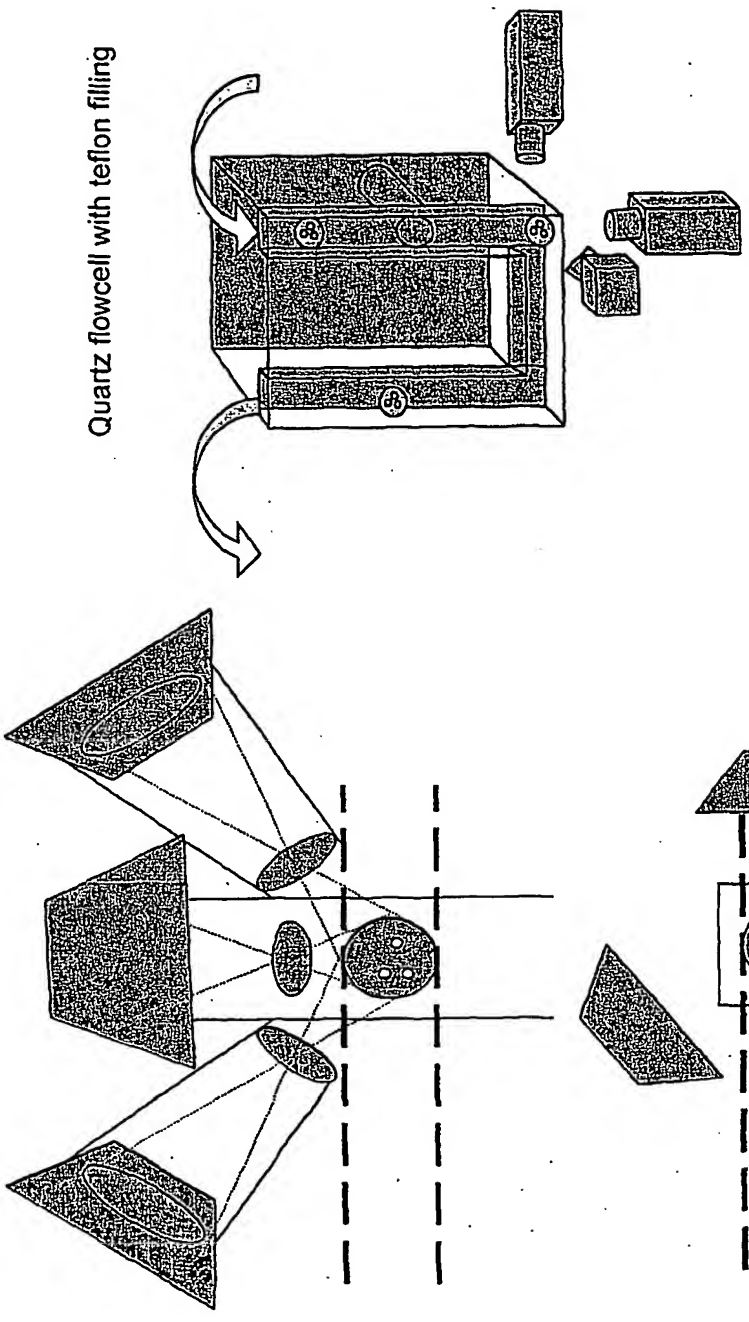

Spatial encoding of beads
Y1,Z1; Y2,Z2; Y3,Z3
X1,Z1; X2,Z2; X3,Z3
X1,Y1; X2,Y2; X3,Y3
X1, Y1, Z1
X2, Y2, Z2
X3, Y3, Z3
V1= (V1X, V1Y, V1Z) = X1-X2, Y1-Y2, Z1-Z2
V2= (V2X, V2Y, V2Z) = X1-X3, Y1-Y3, Z1-Z3
V3= (V2X, V2Y, V2Z) = X2-X3, Y2-Y3, Z2-Z3
CCD's
Scanning
Lasers and PMT
X
Z
Y
PMT
D1= (V1X²+V1Y²+V1Z²)½
D2= (V2X²+V2Y²+V2Z²)½
D3= (V3X²+V3Y²+V3Z²)½

Fig. 2

CCD detection



CDD detection system: The 3 CCD detector plates are positioned on orthogonal axis in Cartesian co-ordinate system around the flow cell

Fig. 3

Recording of coordinates of particles in a bead using focal or confocal microscopy

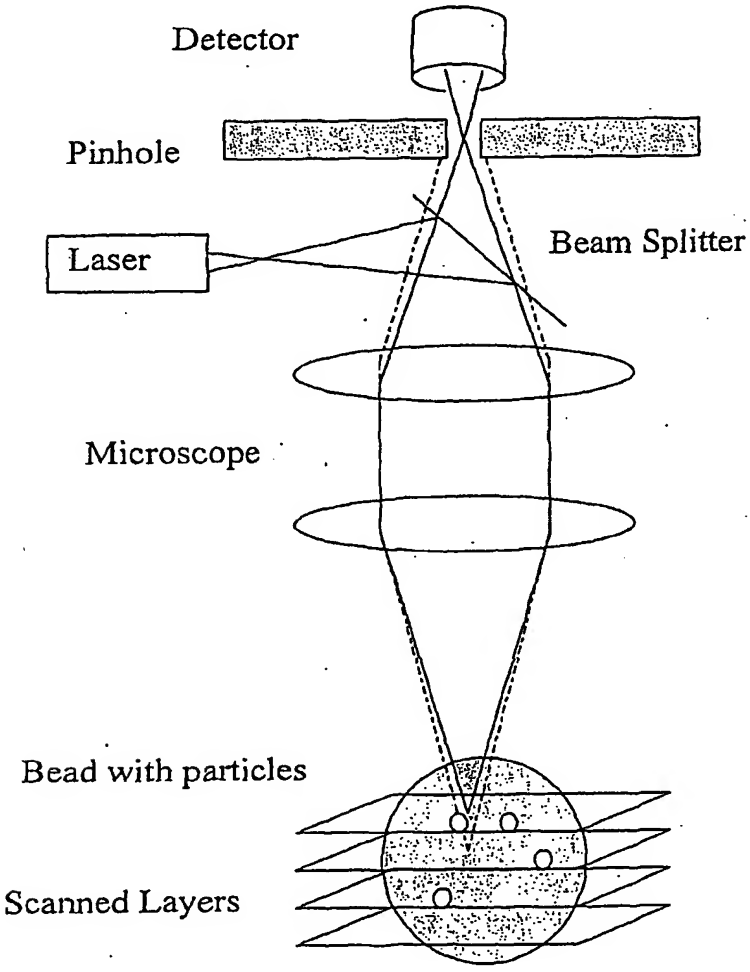


Fig. 4

Recording of coordinates of particles in a moving bead by two alternating scanning lasers

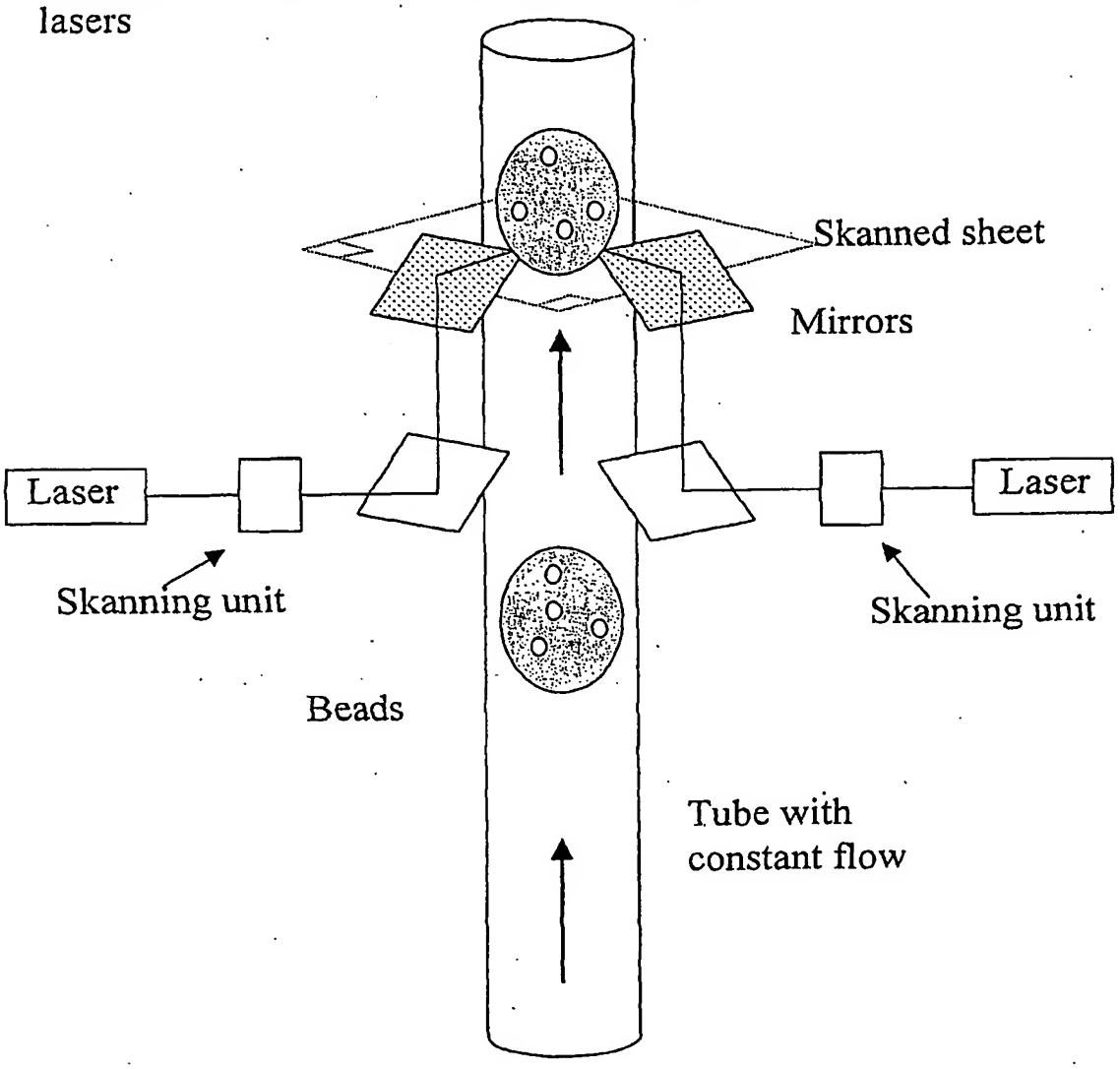


Fig. 5a

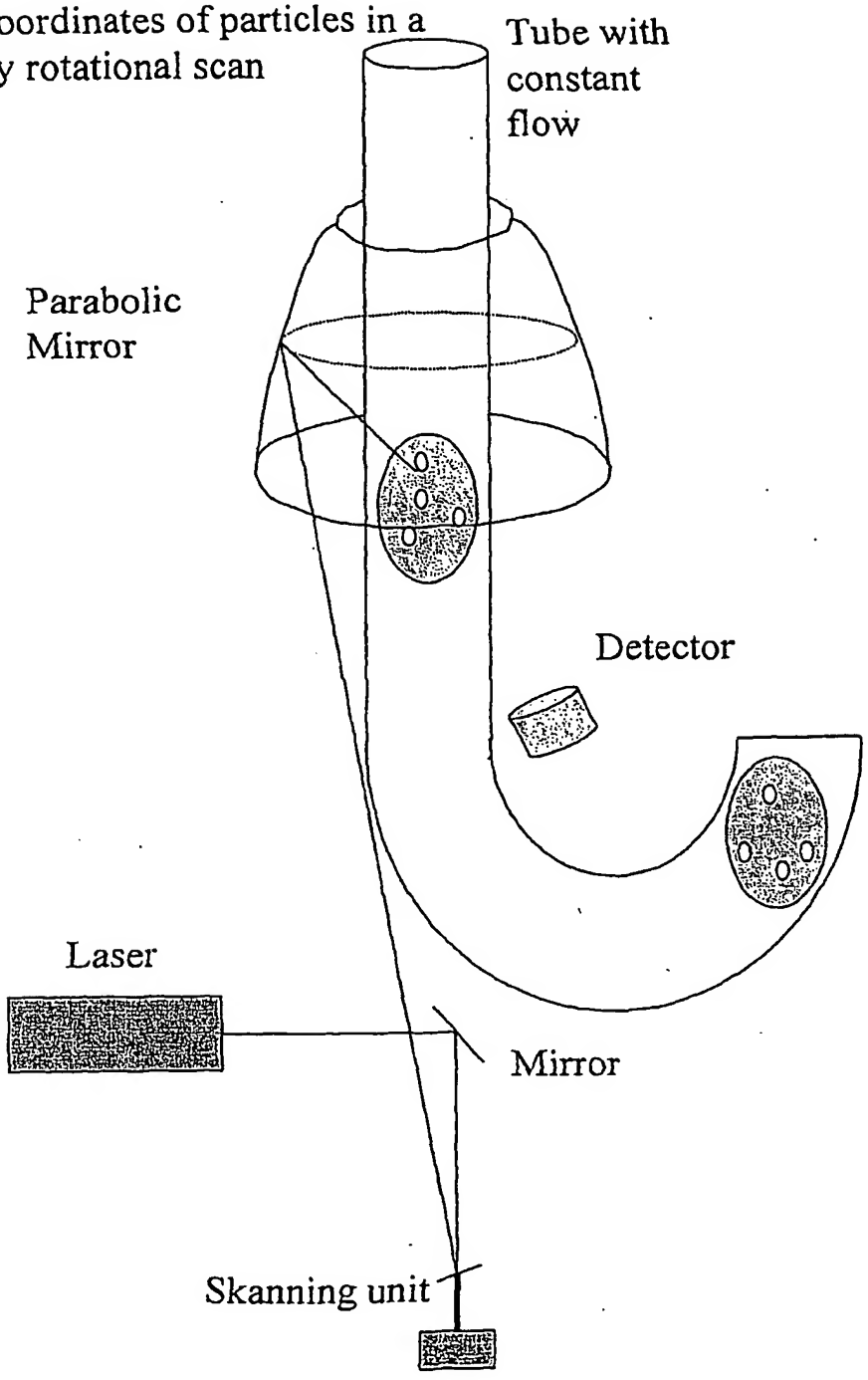
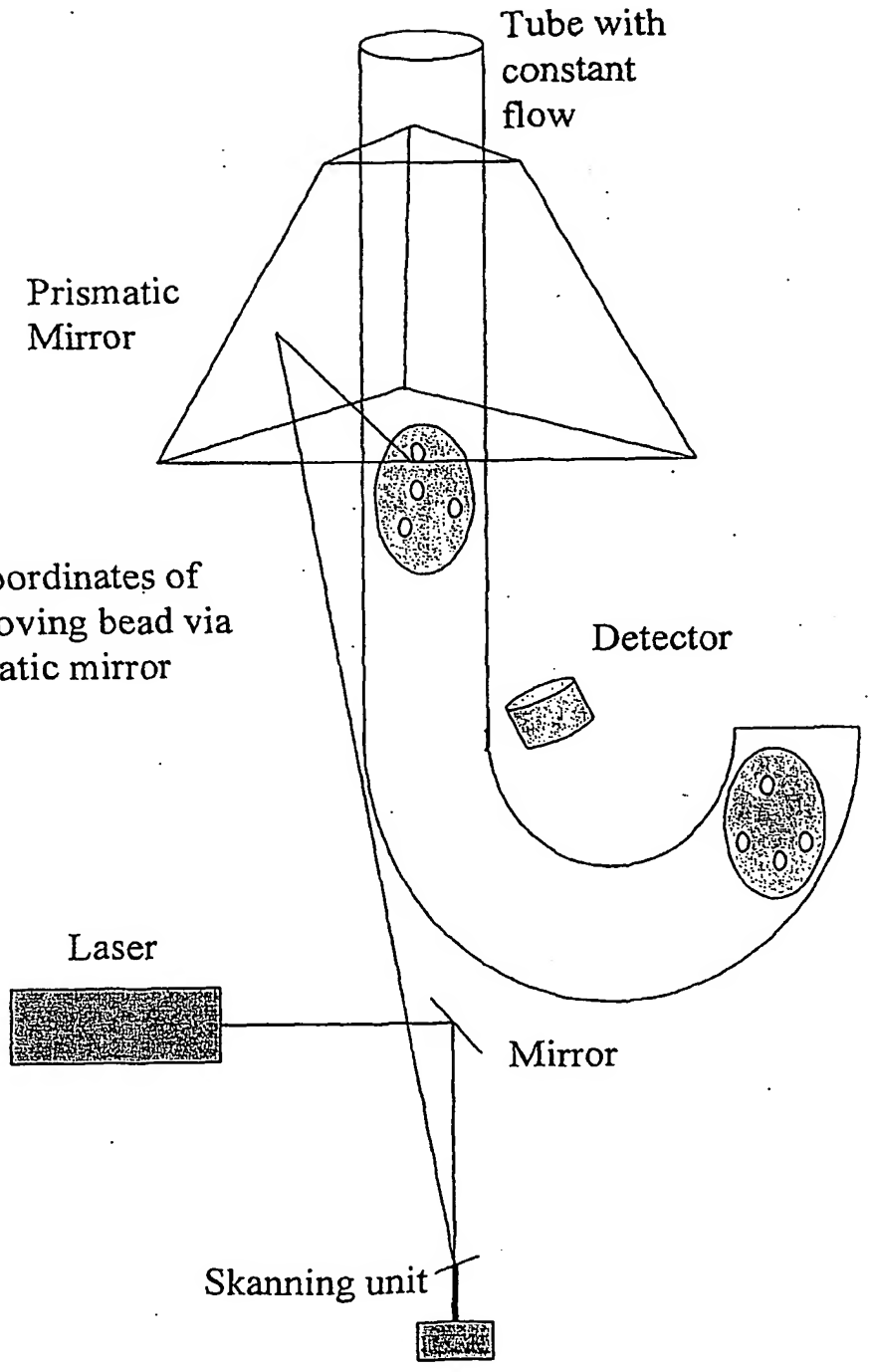

Recording of coordinates of particles in a moving bead by rotational scan
Tube with constant flow
Parabolic Mirror
Detector
Laser
Mirror
Skanning unit

Fig. 5b


Tube with constant flow
Prismatic Mirror
Recording of coordinates of particles in a moving bead via scan on a prismatic mirror
Detector
Laser
Mirror
Skanning unit

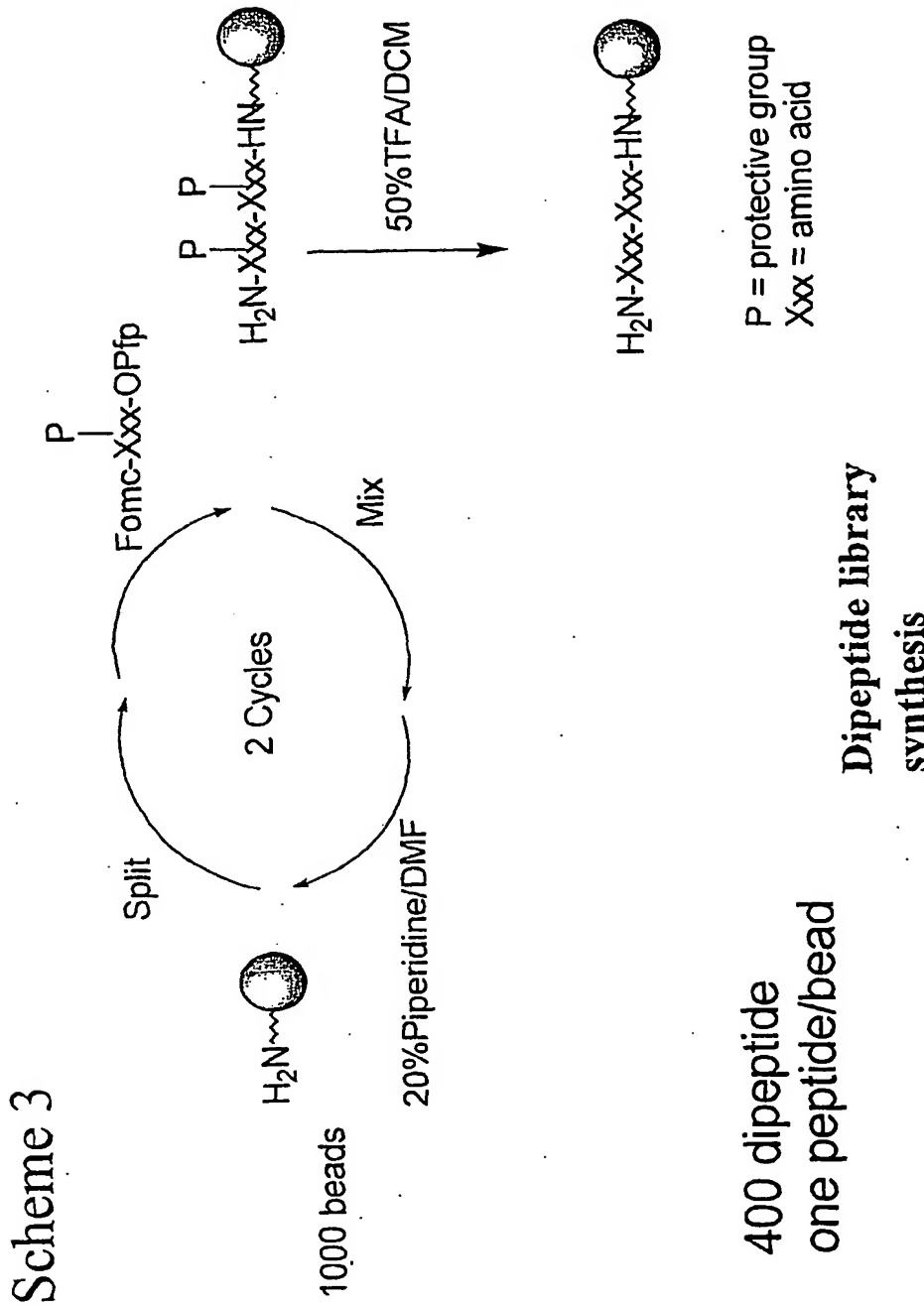
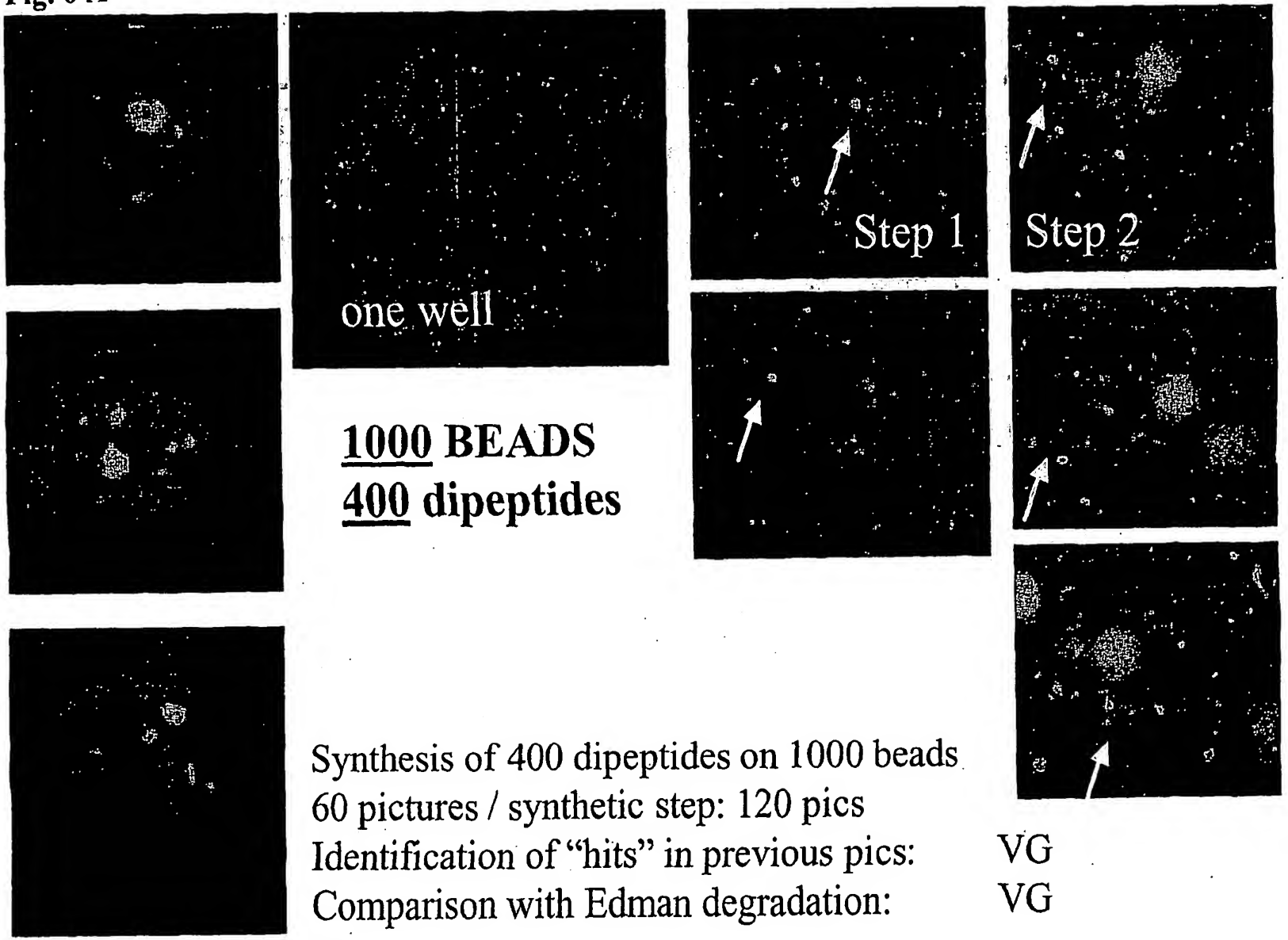

Scheme 3
1000 beads
H_2N
Split
2 Cycles
Fomc-Xxx-OPfp
P
Mix
20%Piperidine/DMF
400 dipeptide
one peptide/bead
H_2N-Xxx-Xxx-HN
P P
50%TFA/DCM
H_2N-Xxx-Xxx-HN
P = protective group
Xxx = amino acid
Dipeptide library
synthesis

Fig. 6 A **Visual decoding of dipeptide library**



Synthesis of 400 dipeptides on 1000 beads
60 pictures / synthetic step: 120 pics
Identification of "hits" in previous pics: VG
Comparison with Edman degradation: VG

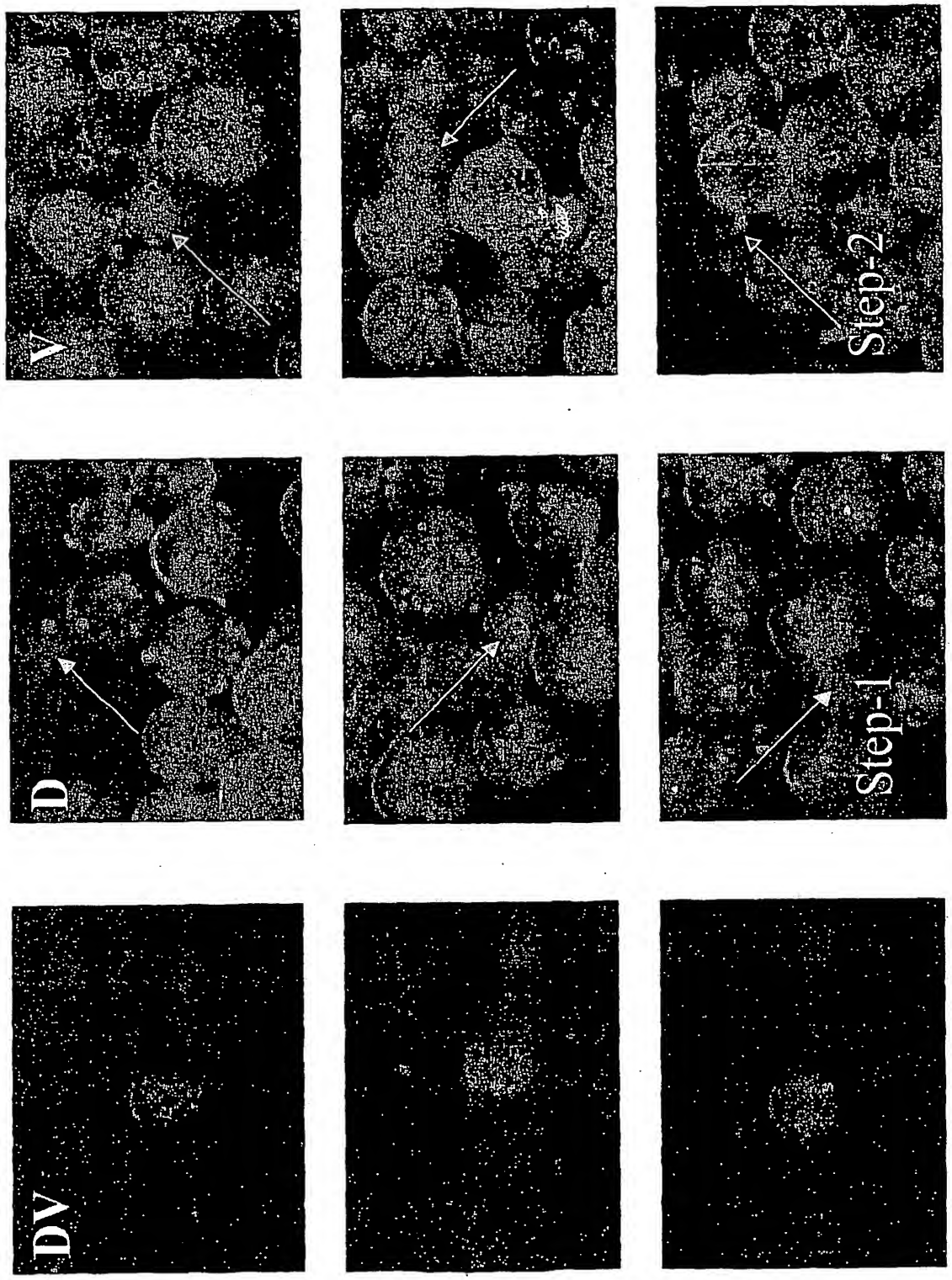


Fig. 6 B

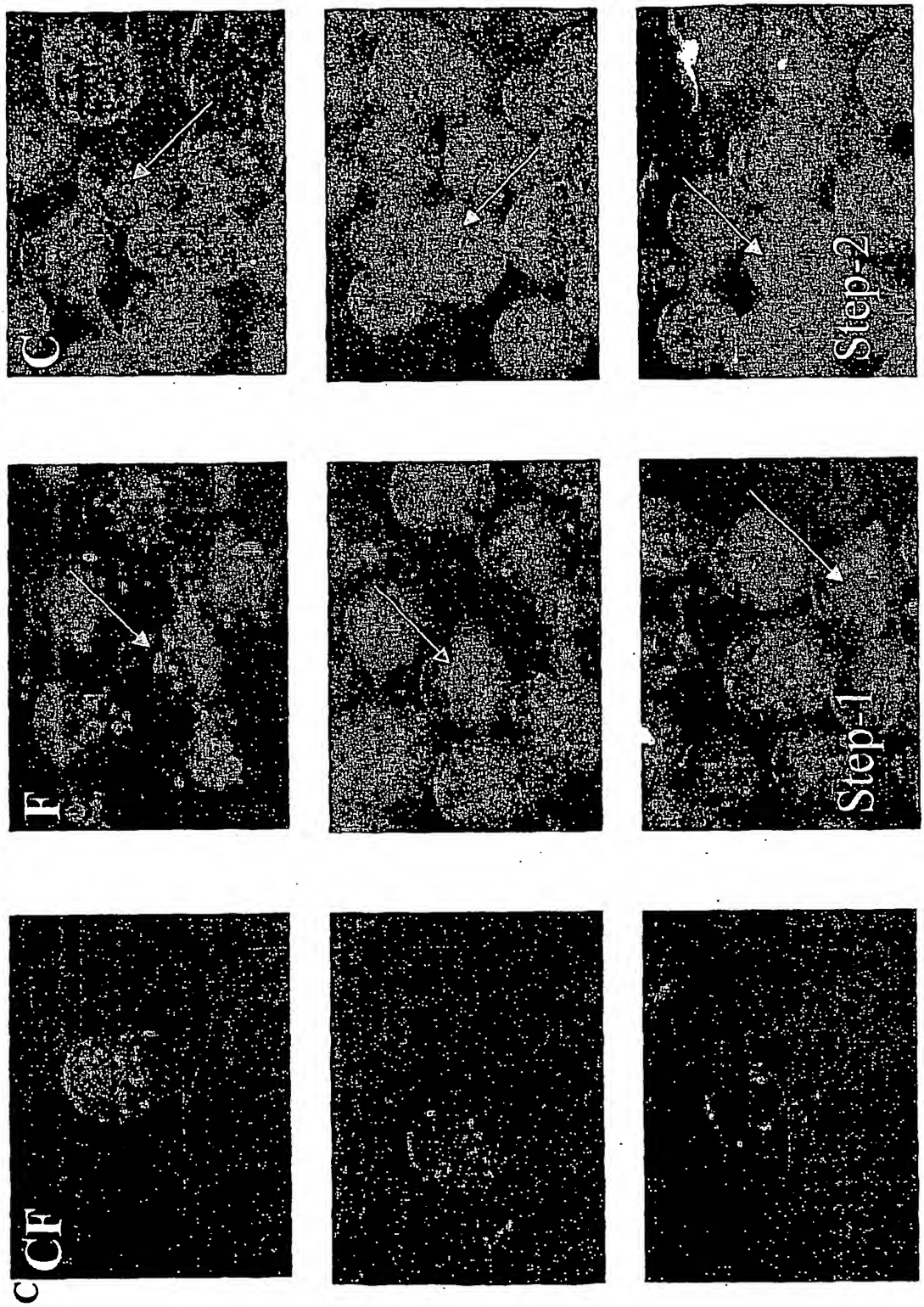


Fig. 6 C

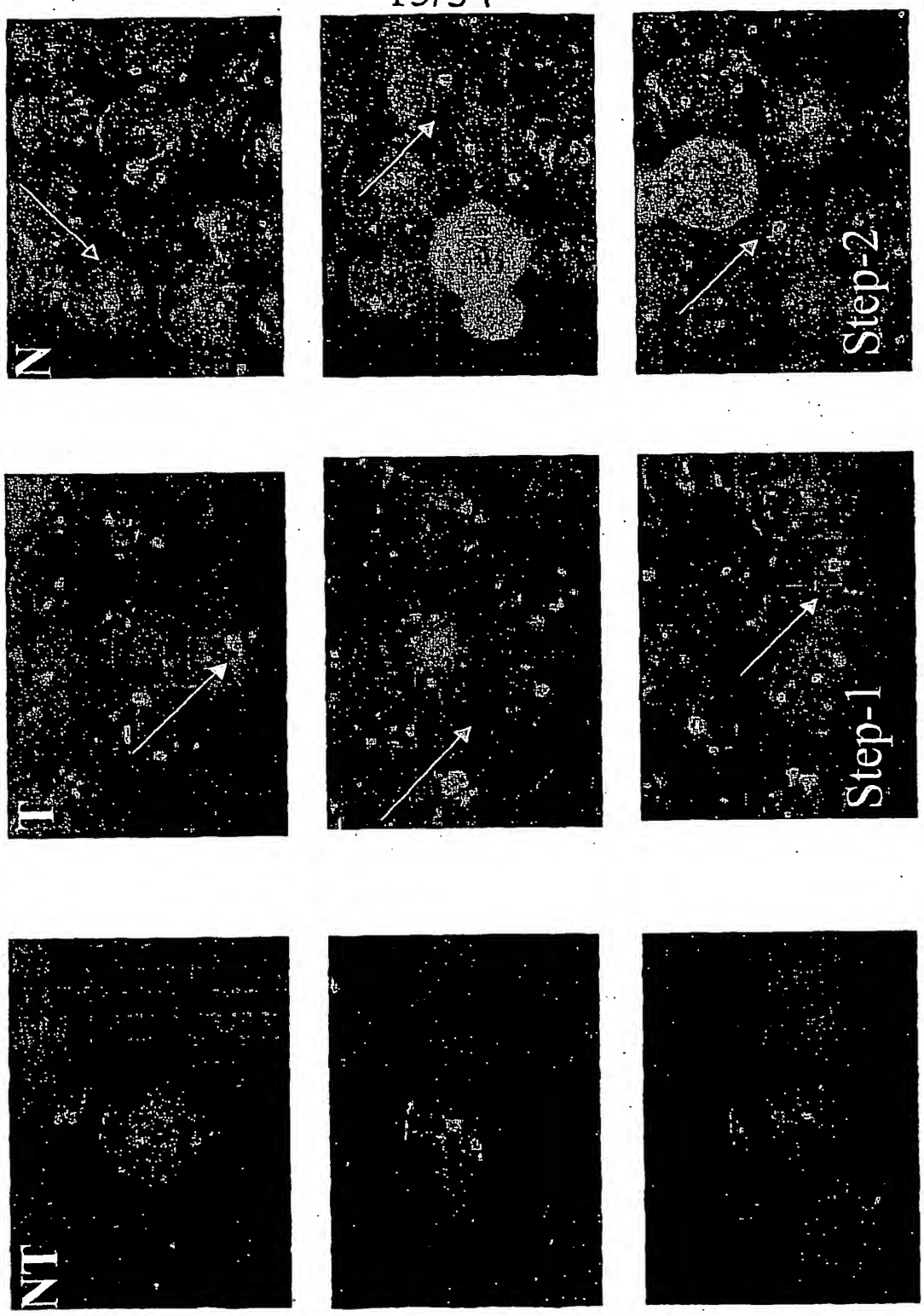


Fig. 6 D

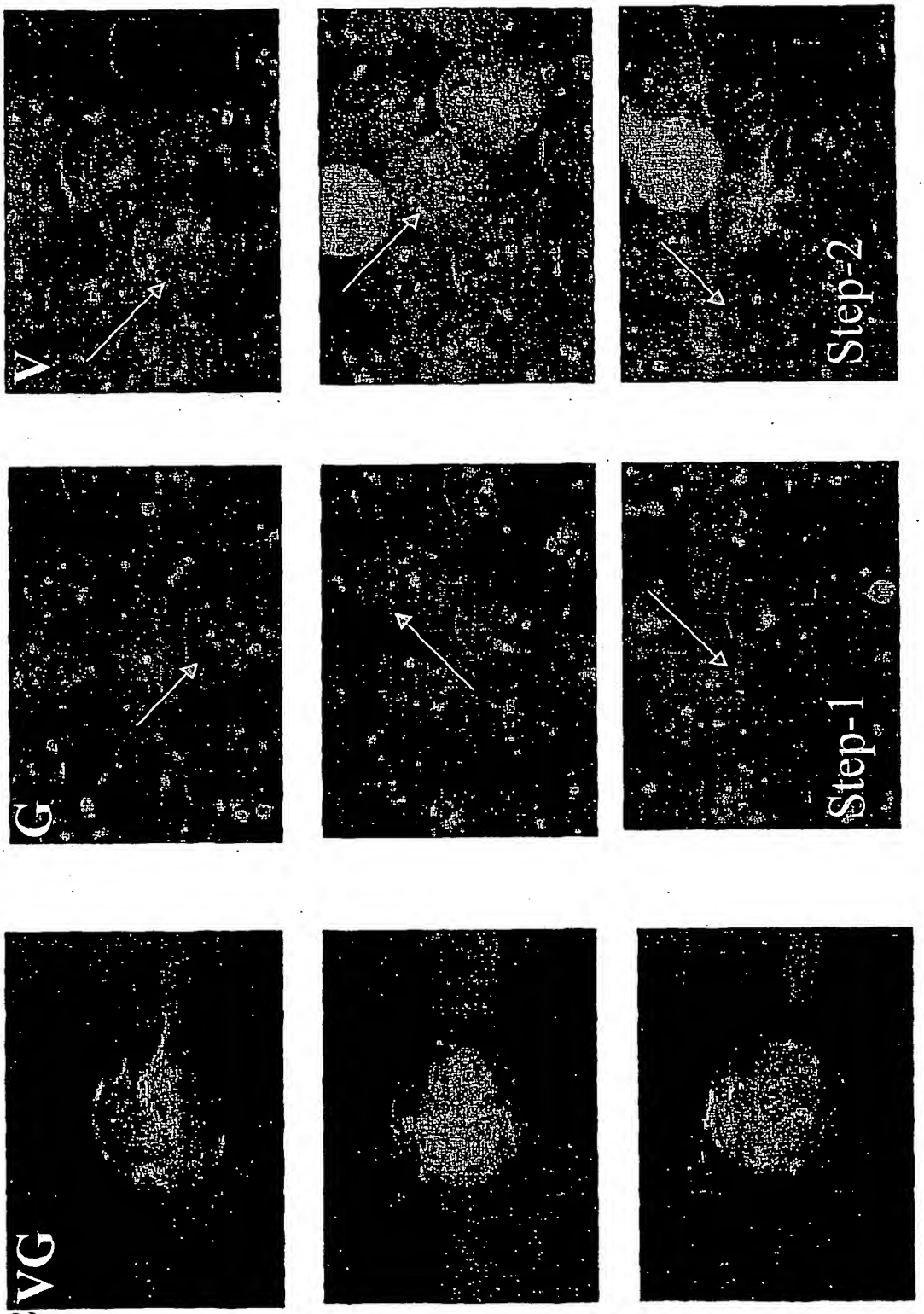


Fig. 6 E

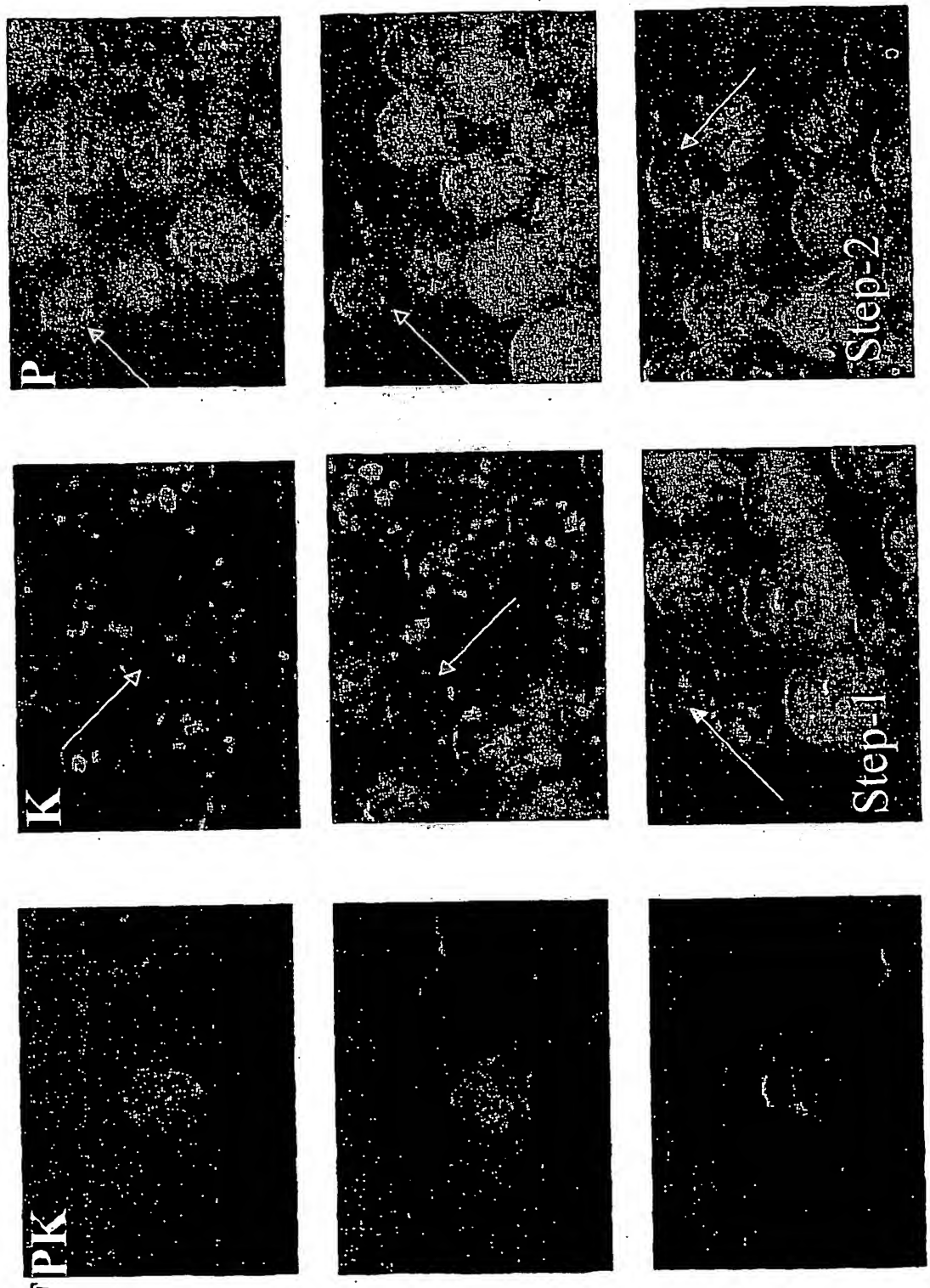


Fig. 6 F PK

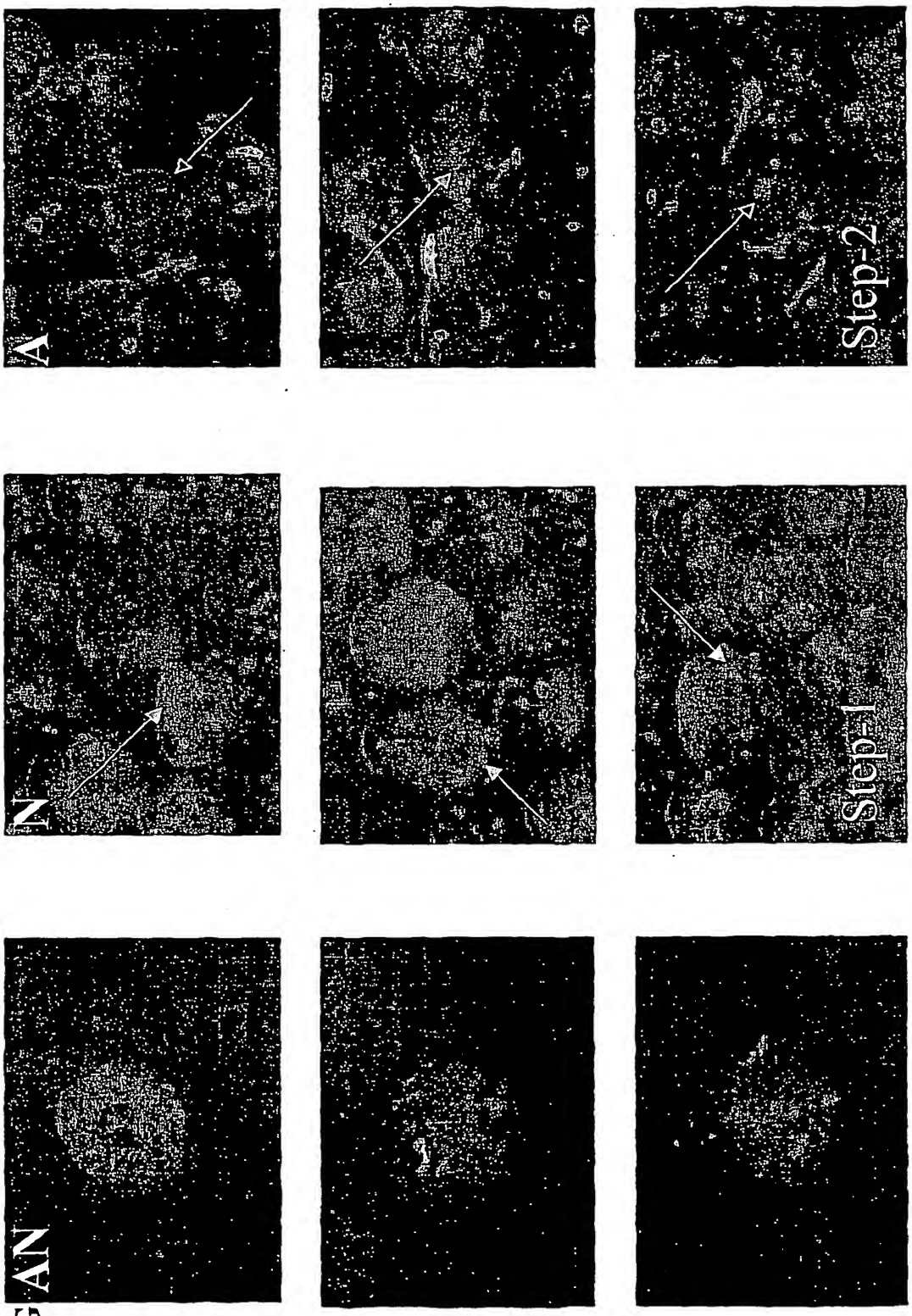


Fig. 6

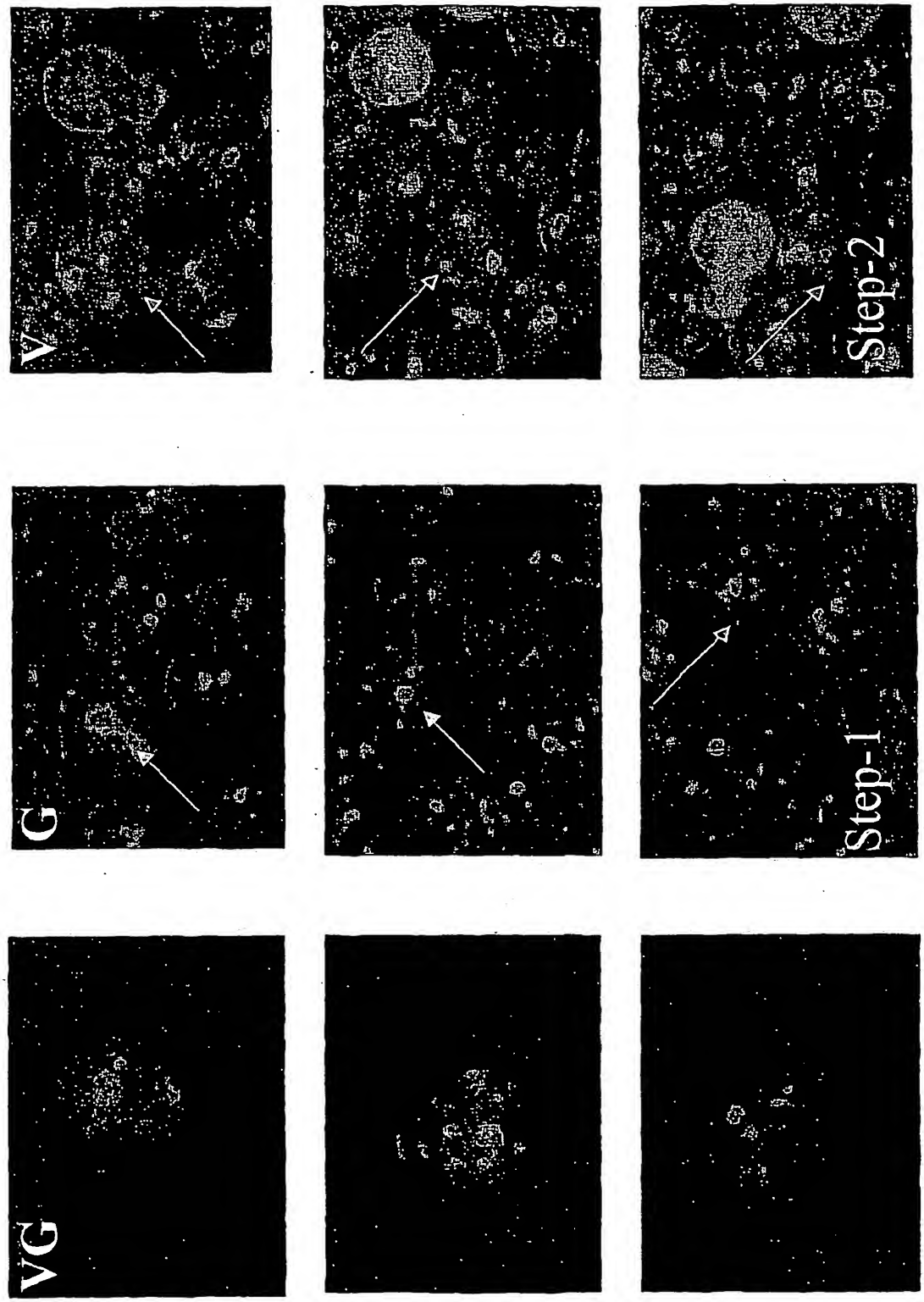


Fig. 6 H

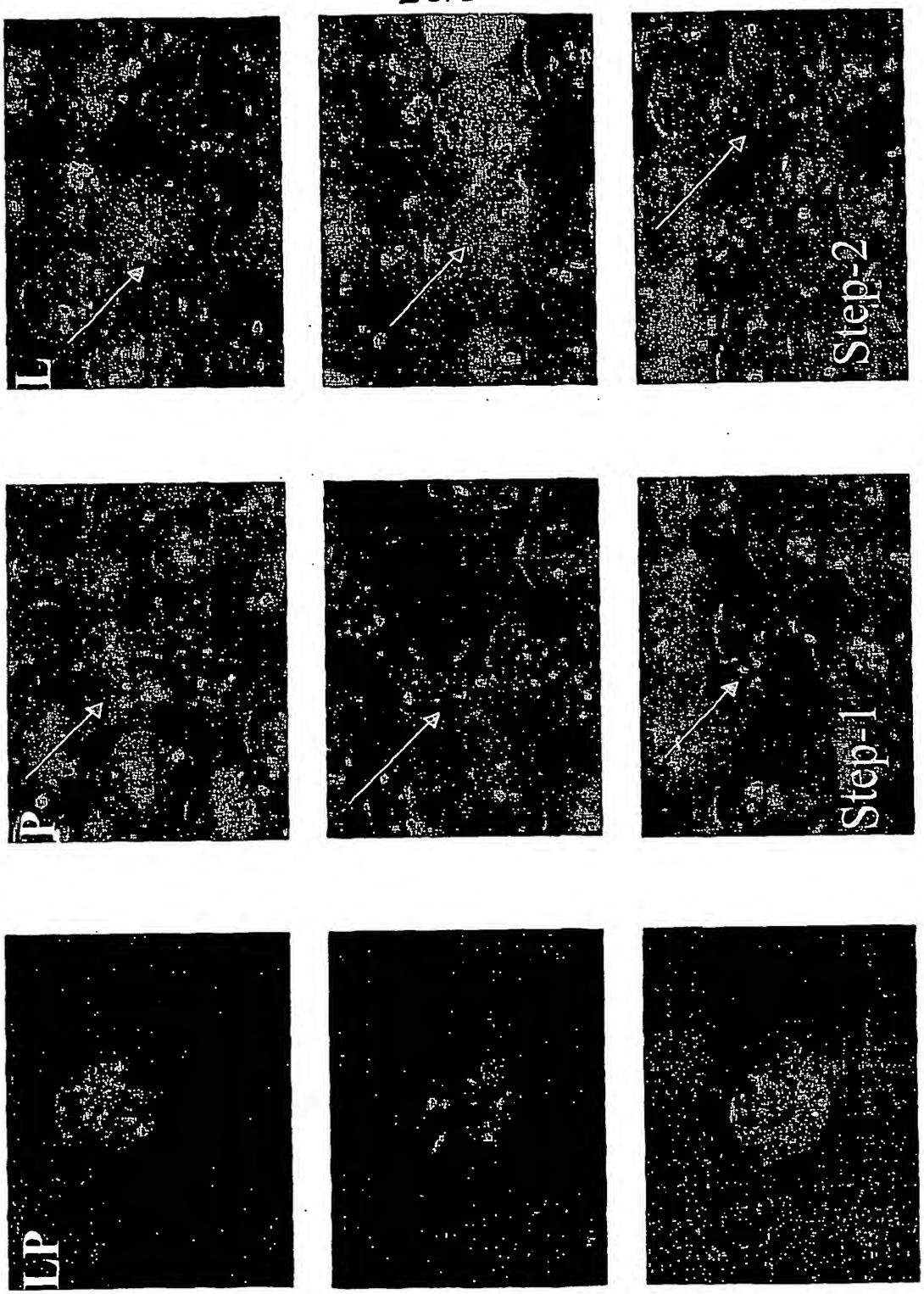


Fig. 6 I

Table 1 **Visual decoding of 20 beads from a library containing 400 dipeptides**

	Seq	Pic	Other possible	
1	CF	CF		
2	AT	A**Y**		
3	DV	DV		
4	EV	E**F**		
5	FR	F**D**		
6	GM	GM	GF	
7	IV	IV	IP	
8	LP	LP		
9	WG	W**W**		
10	YW	YW		

	Seq	Pic	Other possible	
11	HF	HL		
12	NT	NT		
13	AN	AN		
14	PH	P**F**		
15	PK	PK		
16	FR	FR	FH	VR
17	VG	VG		
18	RQ	RQ	VQ	
19	VG	VG		
20	AL	AL	VL	AI

Fig. 7A 3 Orthogonal pictures

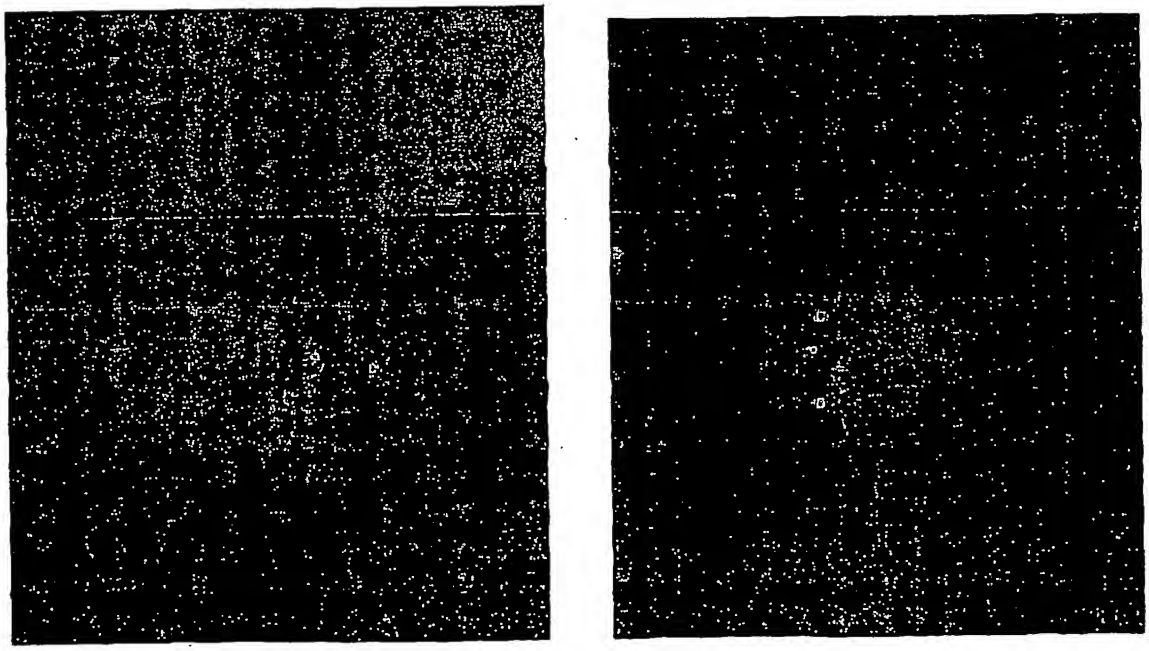
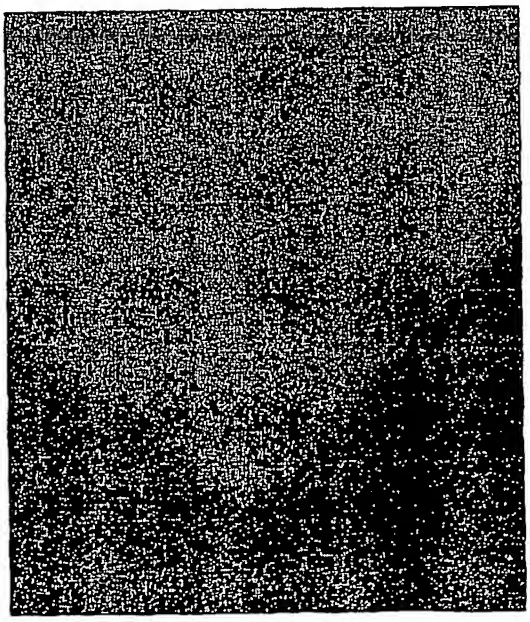
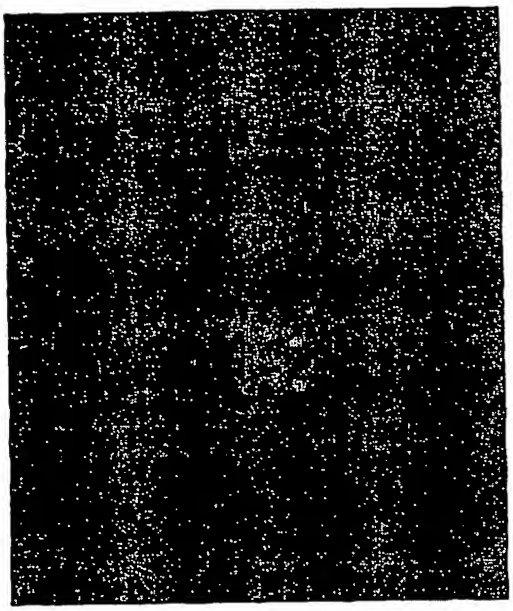


Fig. 7 B 3 Orthogonal pictures



0,0 0,0 0,0
X1, X2 X1, X2 Y1, Y2
Y1, Y2 Z1, Z2 Z1, Z2



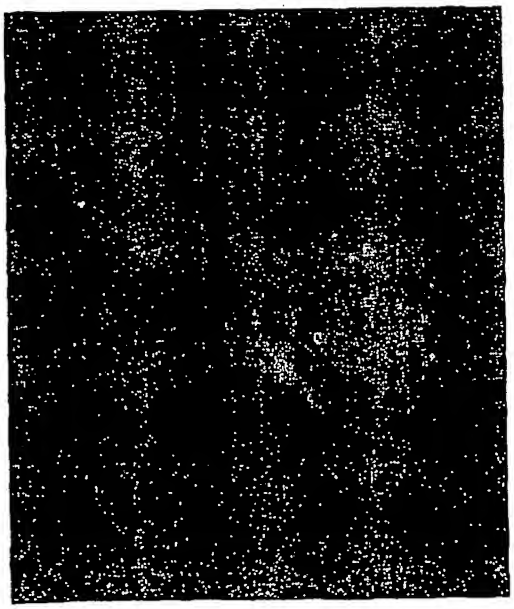
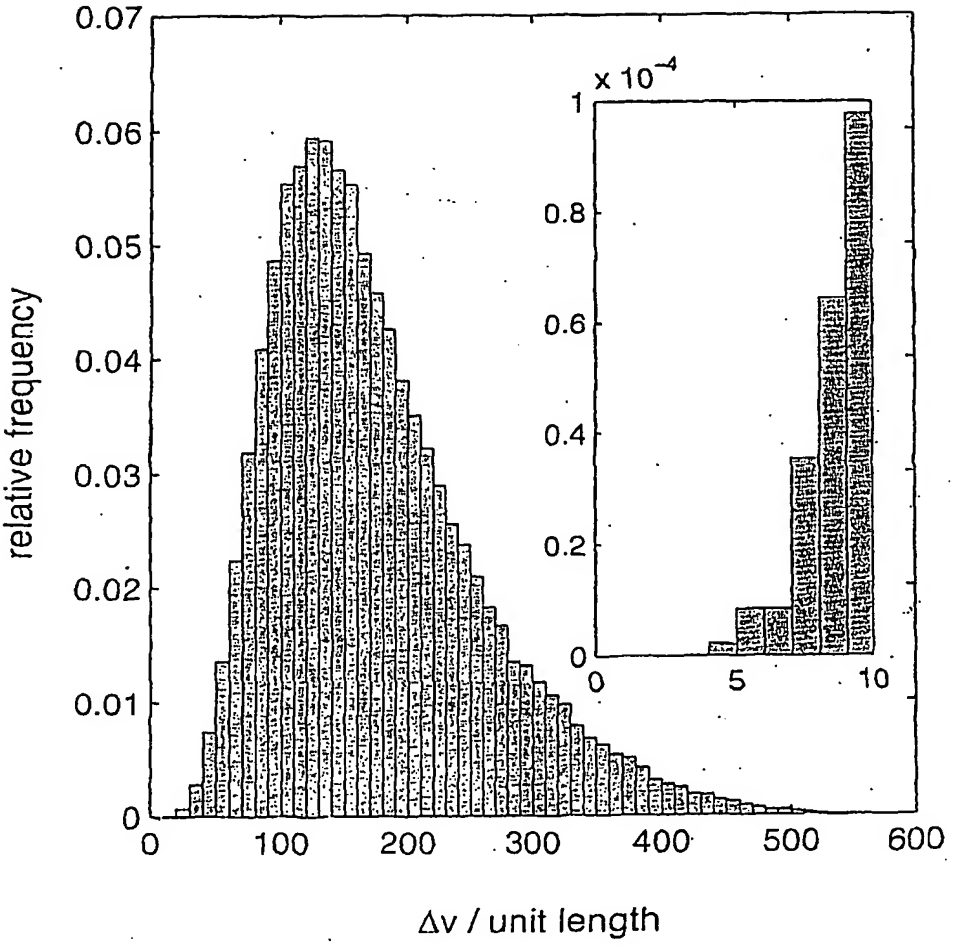


Fig. 8


relative frequency
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
100
200
300
400
500
600
Δv / unit length
x 10^-4
1
0.8
0.6
0.4
0.2
0
0
5
10

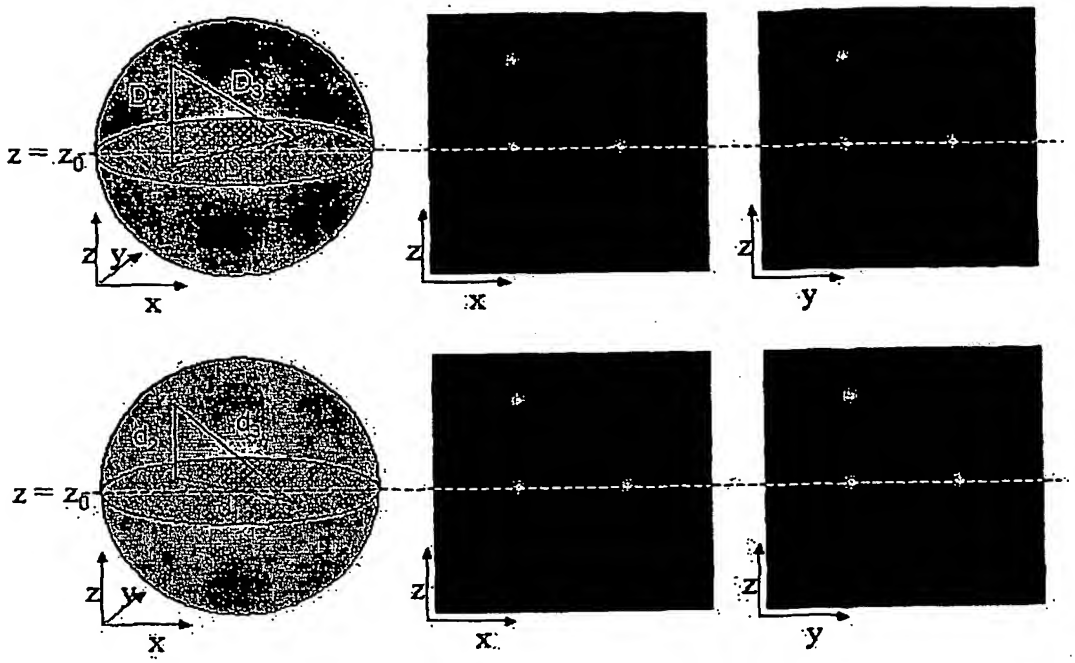


Fig. 9

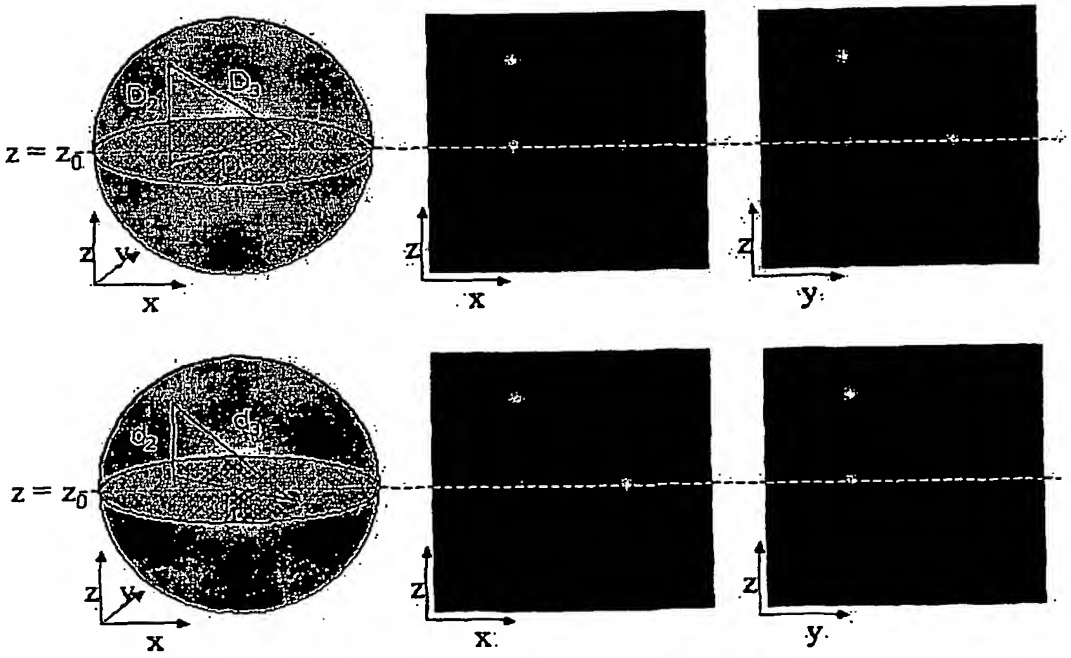
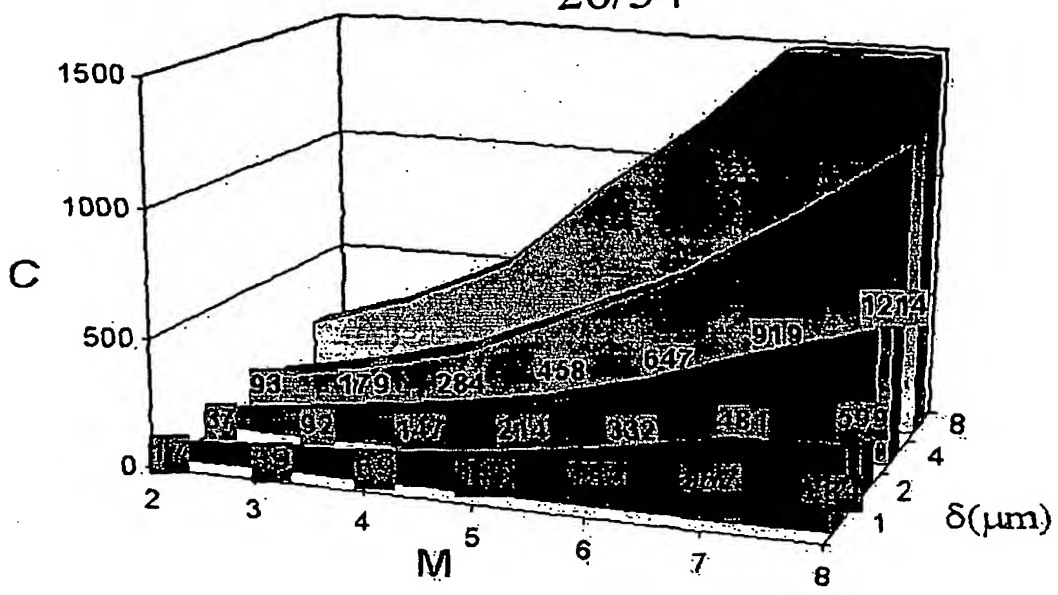


Fig. 10


1500
1000
C
500
0
2
3
4
5
6
7
8
M
1
2
4
8
δ(μm)
1214
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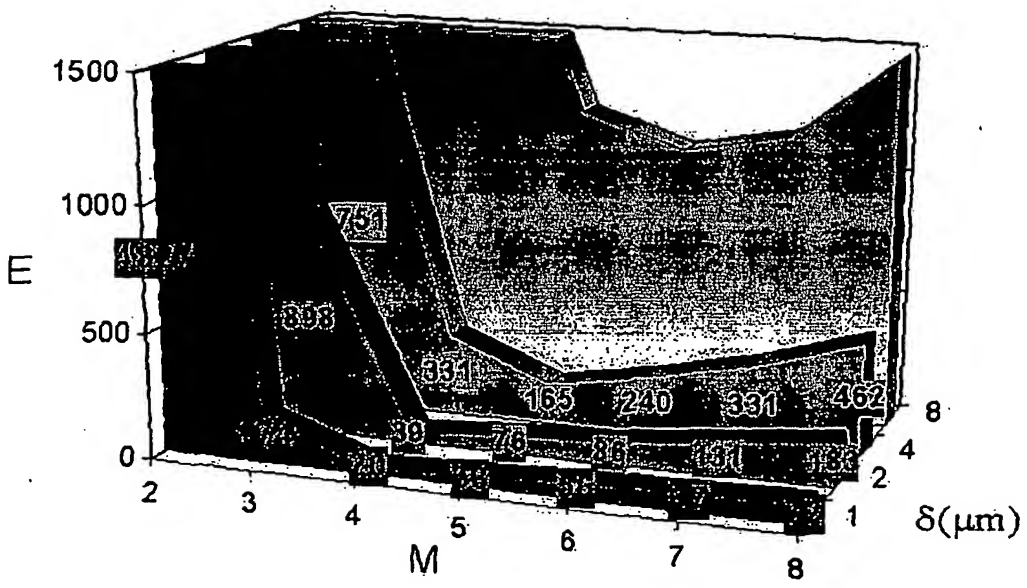
647
458
284
179
93


1500
1000
E
500
0
2
3
4
5
6
7
8
M
1
2
4
8
δ(μm)
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808
331
165
240
331
462
39
76
86

Fig. 11

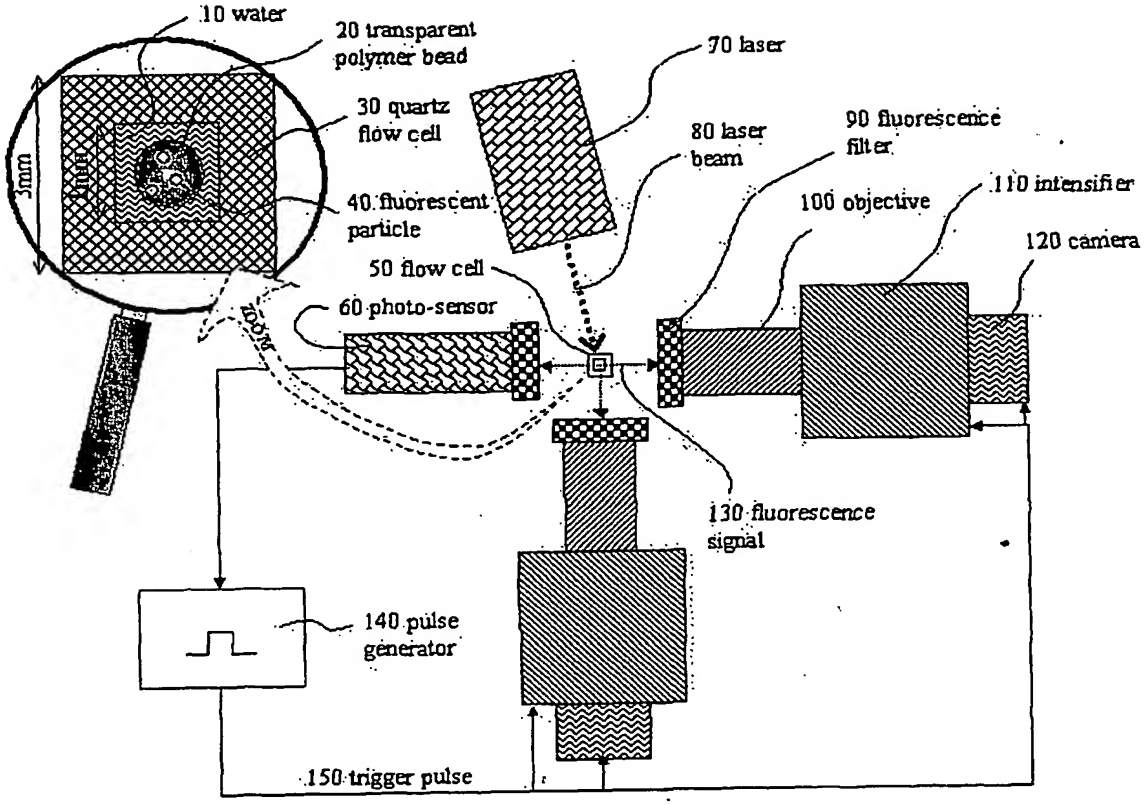

10 water
20 transparent polymer bead
30 quartz flow cell
40 fluorescent particle
50 flow cell
60 photo-sensor
70 laser
80 laser beam
90 fluorescence filter
100 objective
110 intensifier
120 camera
130 fluorescence signal
140 pulse generator
150 trigger pulse
3mm
1mm
200µm

Fig.12

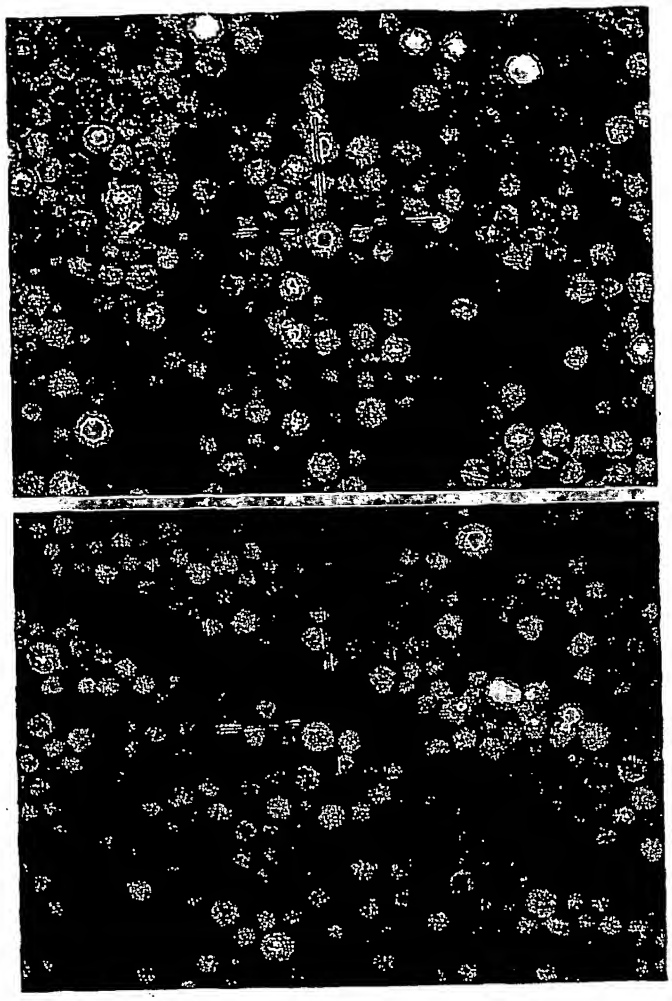
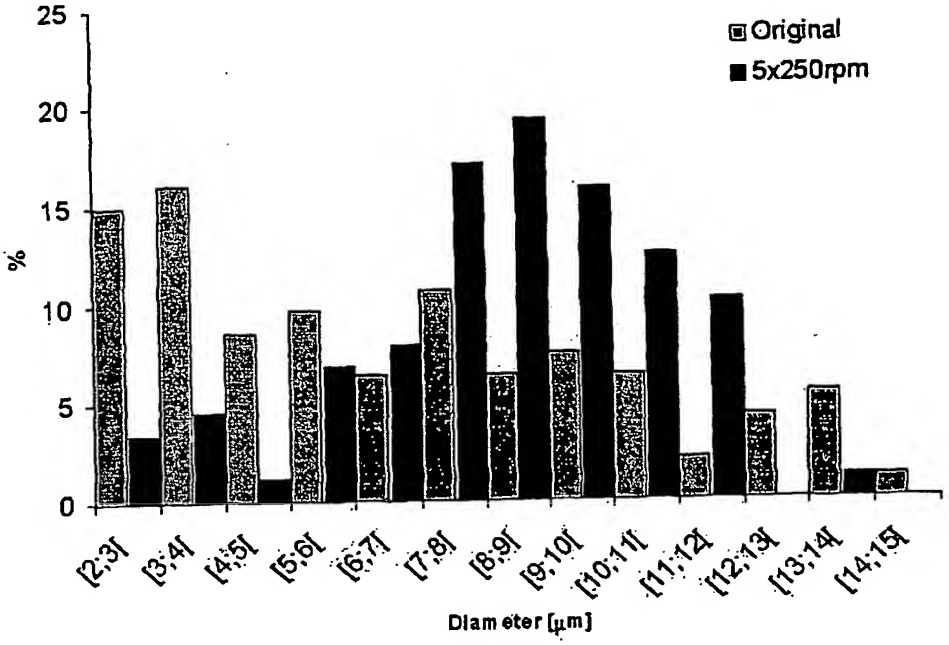


Fig. 13


Original
5x250rpm
25
20
15
10
5
0
%
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[3;4[
[4;5[
[5;6[
[6;7[
[7;8[
[8;9[
[9;10[
[10;11[
[11;12[
[12;13[
[13;14[
[14;15[
Diameter [µm]

Fig. 14

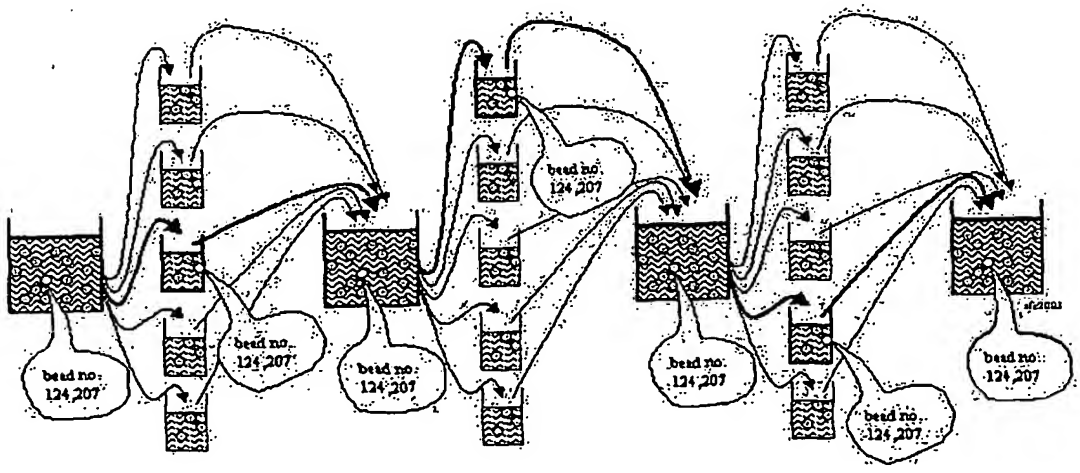

bead no.
124,207

Fig. 15

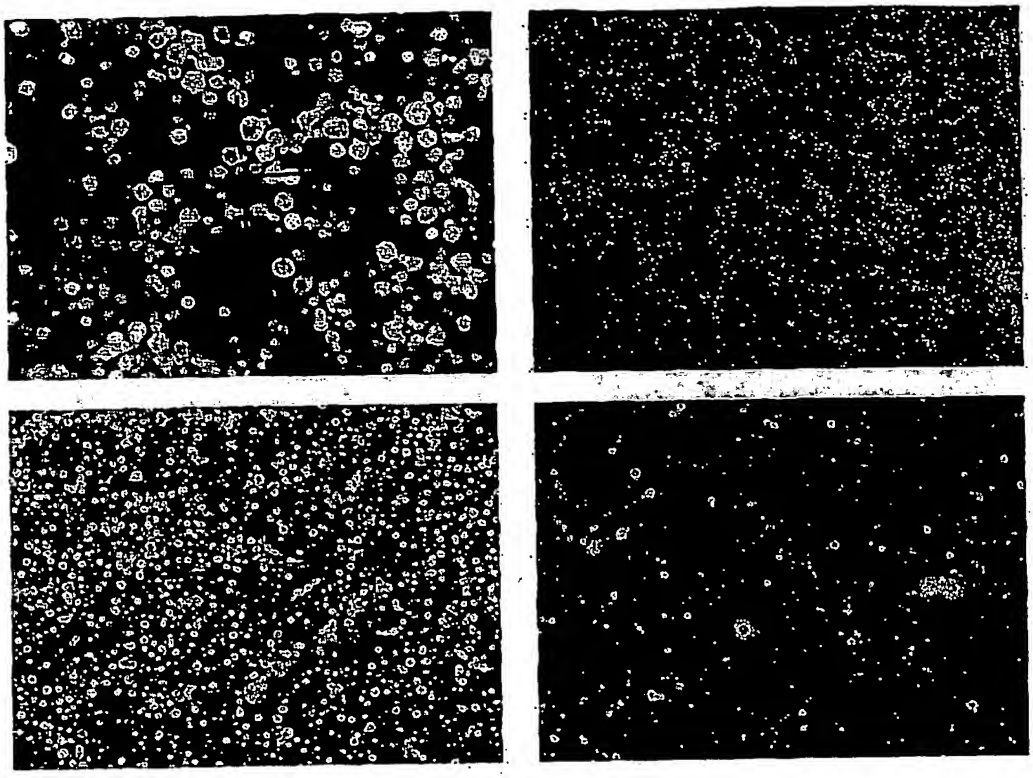


Fig. 16

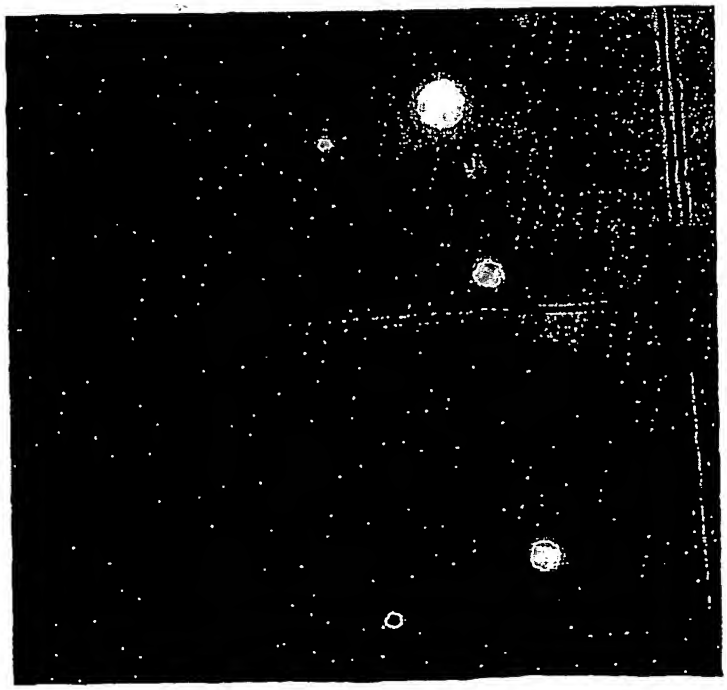


Fig. 17

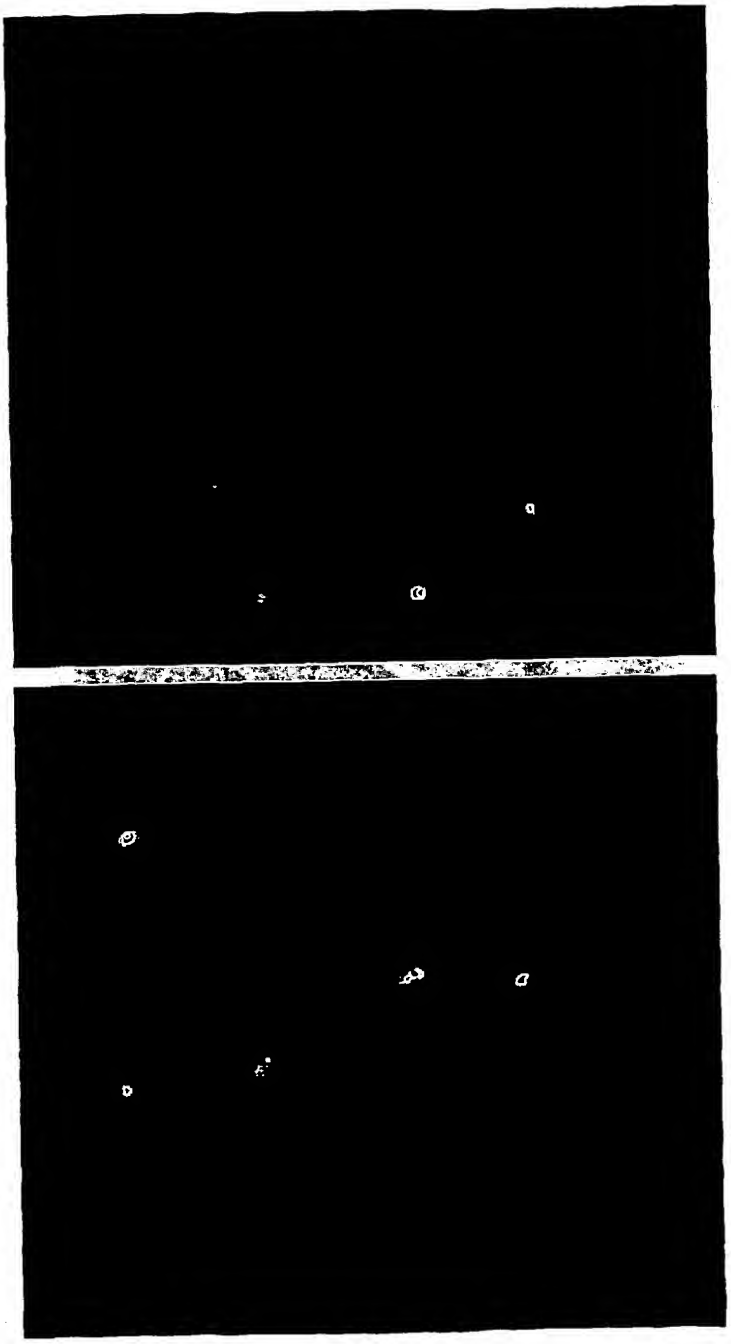
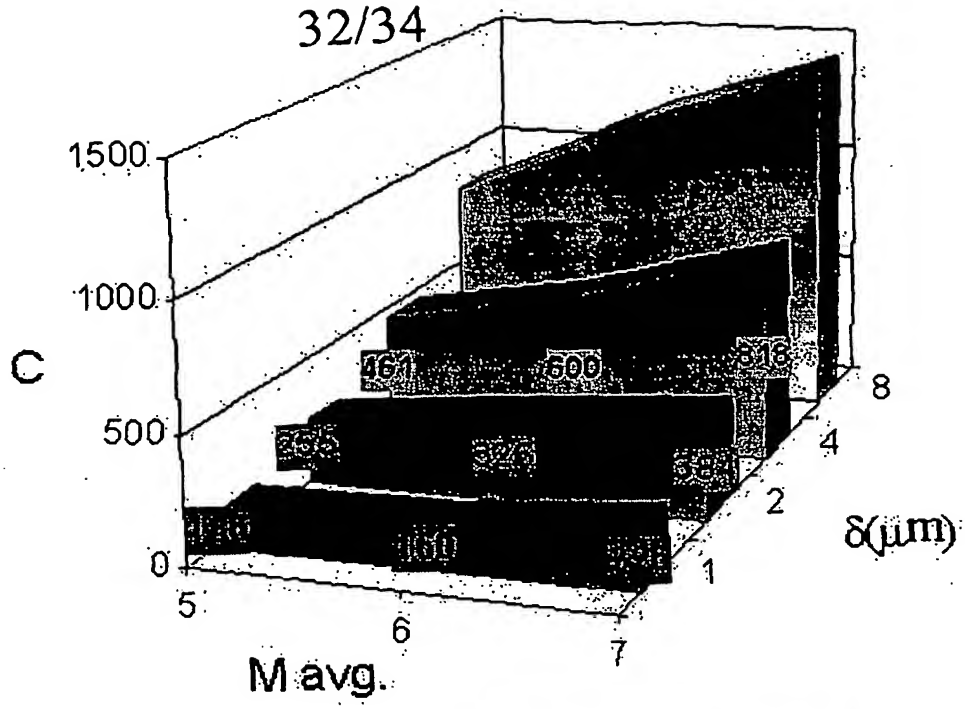


Fig. 18


1500
1000
500
0
C
461
600
316
5
6
7
M avg.
1
2
4
8
δ(μm)

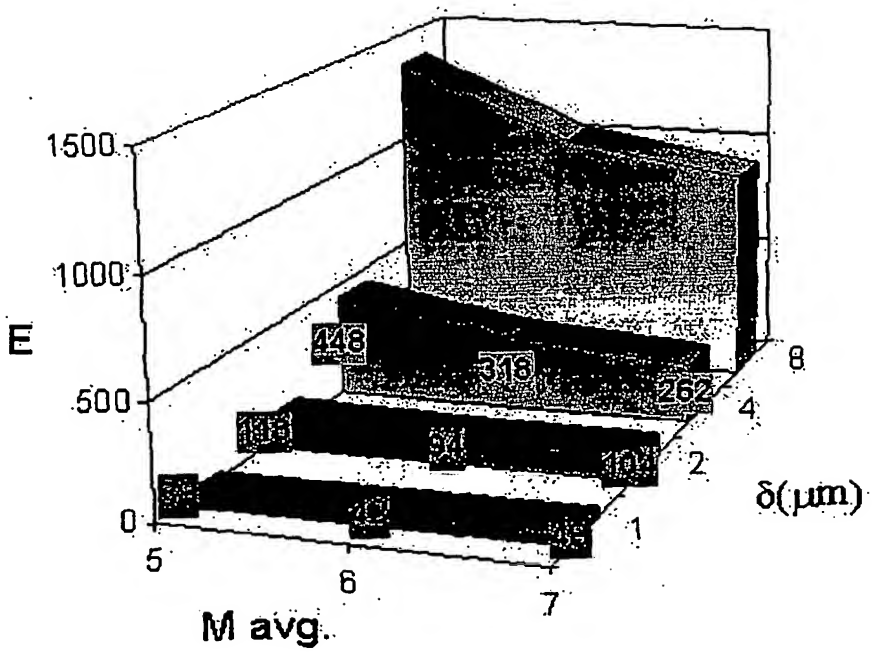

1500
1000
500
0
E
448
318
262
5
6
7
M avg.
1
2
4
8
δ(μm)

Fig. 19

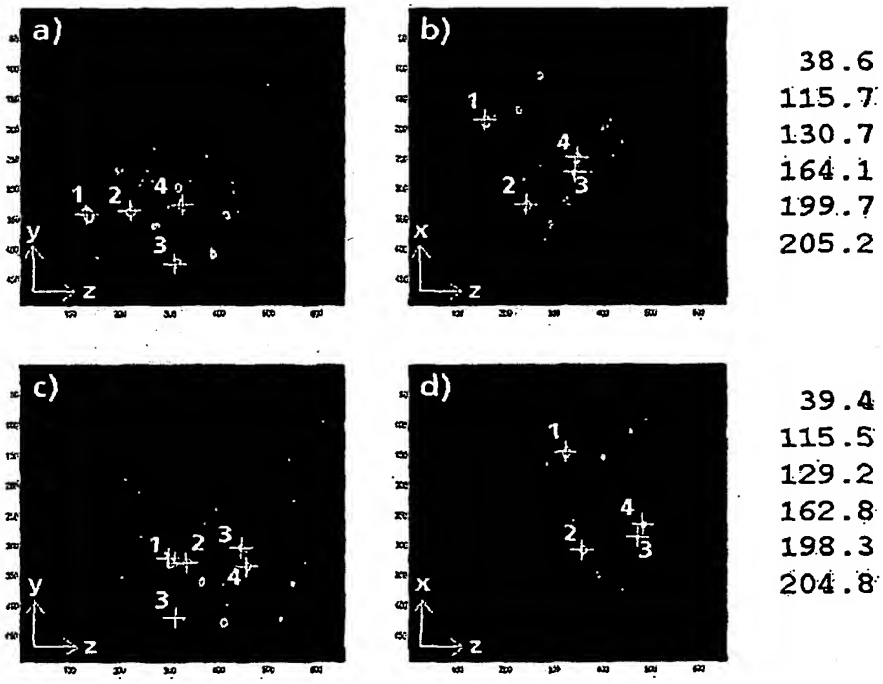

a)
b)
c)
d)
1
2
3
4
y
x
z
38.6
115.7
130.7
164.1
199.7
205.2
39.4
115.5
129.2
162.8
198.3
204.8

Fig. 20

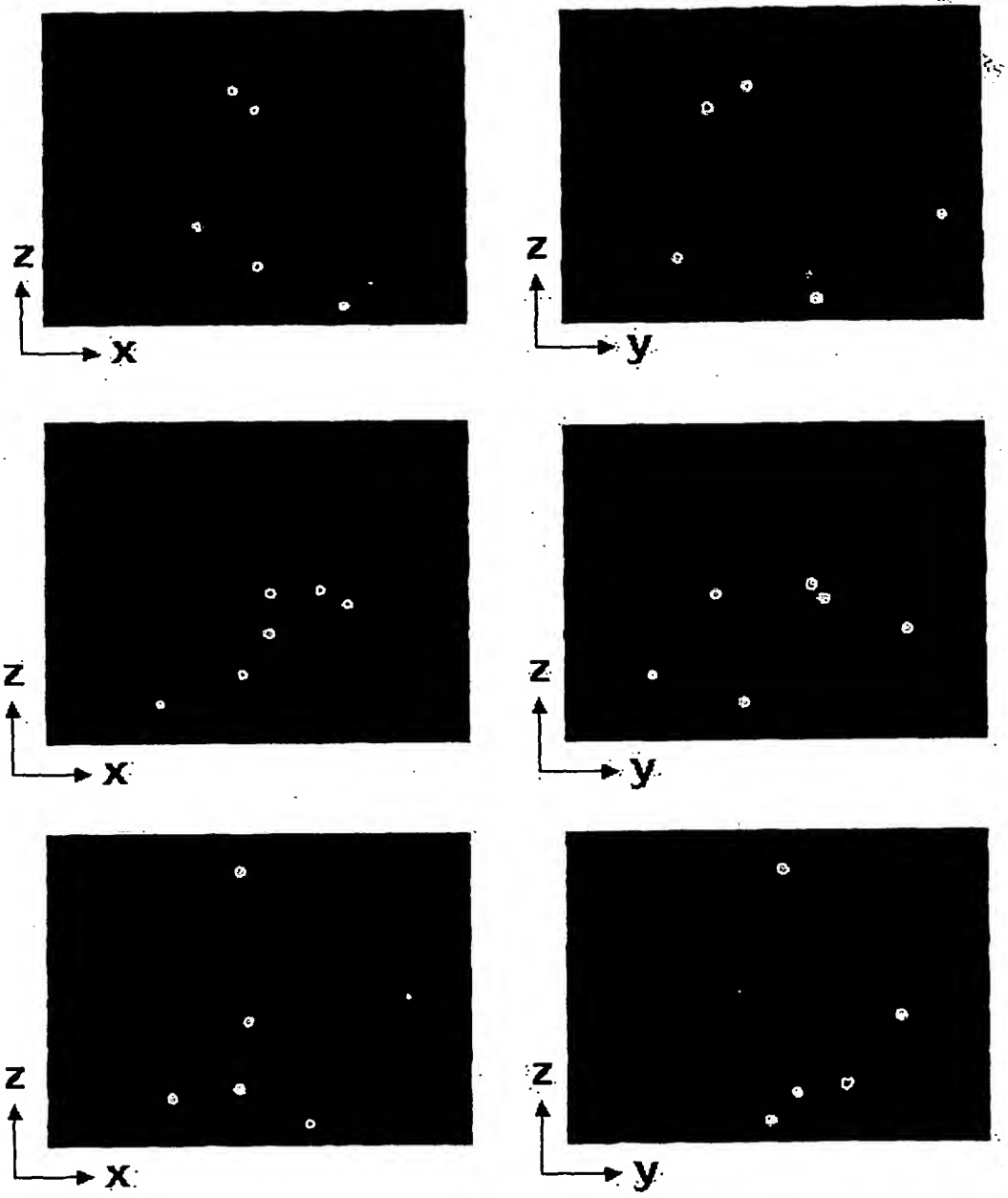


Fig. 21